USER
MANUAL

Version 2.0 for Microsoft® Windows

MasterPlex™ QT
Multiplex Data Analysis Software

MiraiBio

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For Research Use Only

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MiraiBio

MasterPlex™ QT 2.0

Analysis software for multiplex data from the Luminex™ 100 system.

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CHAPTER 1

WELCOME

MiraiBio MasterPlex™ QT 2.0

Welcome to the MiraiBio MasterPlex™ QT User Manual. MasterPlex QT software analyzes results files (.csv) from the Luminex™ 100 system.*

1.1 About This Manual

This manual explains how to use the MasterPlex QT 2.0 software to:

- import results files (*.csv) from the Luminex 100 system
- designate standard, unknown, control, and background wells
- generate standard curves
- compute analyte concentrations
- generate data charts and reports

What's New in MasterPlex QT 2.0

MasterPlex QT 2.0 offers new features, including the ability to:

- apply a standard from one plate (source plate) to compute the concentrations in another plate (destination plate)
- view a residual plot for standard curves, specify outliers in a standard curve, or apply weighting to a standard curve
- use unknown samples that are diluted prior to the assay and analysis
- create a *virtual plate* (a software simulation of a plate) that contains data from more than one actual plate (.csv or .mlx) so that results across multiple plates can be compared and analyzed
- generate a Dose-Reponse curve and determine the logEC50 value

Conventions Used in This Manual

This manual describes the steps required to perform the various tasks associated with the MasterPlex QT software. The manual uses a step format to explain the various tasks associated with MasterPlex QT. The symbol ⇒ may follow a step instruction. It indicates the software response to the action performed by the user.

Screen Captures

Screen captures may accompany the step instructions for further illustration. The screen captures in this manual may not exactly match those displayed on your screen.

1.2
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CHAPTER 2

INSTALLING MASTERPLEX QT

This chapter explains the minimum hardware and software requirements needed to install and use MasterPlex™ QT 2.0. It provides installation instructions for a computer connected to the Luminex™ 100 system.

2.1 Requirements

For optimum performance, MasterPlex QT requires hardware and software that meet or exceed the following specifications. It is also strongly recommended that you use the Luminex XY platform.

Minimum Hardware Requirements

Platform	PC
CPU	Intel Pentium III 500 MHz or equivalent, Intel Pentium4 800 MHz or better recommended
Memory (RAM)	64 MB RAM or higher for Windows® 98/98SE; 128MB or higher for Windows Me/NT4.0/XP
Storage space (HDD)	15 MB available space for the installation
Input devices	Keyboard and mouse or any other pointing device
Video RAM	4MB or higher
Monitor resolution	XGA (1024x768 pixels or higher; 1280 x1024 recommended)
Monitor color	16-bit color (high color) or higher
CD-ROM drive	Required for CD media version. Not applicable for download version.

Software Requirements

Operating system	Microsoft Windows 98/98SE/Me/NT4.0 SP6/2000/XP (Windows 2000 or XP recommended)

2.2
Installing MasterPlex QT

1. Insert the MasterPlex™ QT CD-ROM in the workstation computer and double-click MasterPlex QT.exe.
   ⇒ The installation process begins and the InstallShield Wizard appears (Figure 2.1).

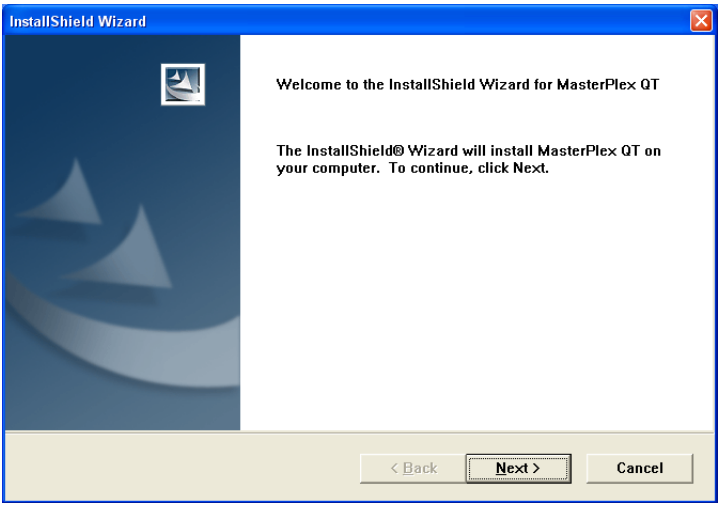

InstallShield Wizard
Welcome to the InstallShield Wizard for MasterPlex QT
The InstallShield® Wizard will install MasterPlex QT on your computer. To continue, click Next.
< Back
Next >
Cancel

Figure 2.1 InstallShield Wizard, Welcome screen

2. To continue the installation, click **Next**.
   ⇒ The Choose Desination Location window appears (Figure 2.2).

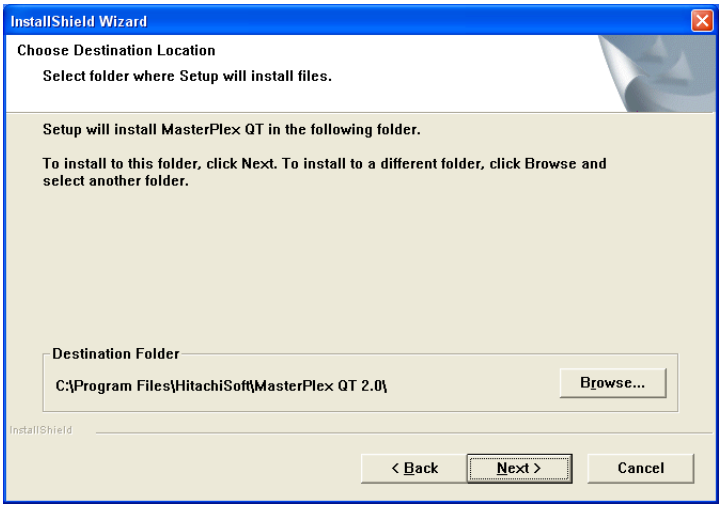

InstallShield Wizard
Choose Destination Location
Select folder where Setup will install files.
Setup will install MasterPlex QT in the following folder.
To install to this folder, click Next. To install to a different folder, click Browse and select another folder.
Destination Folder
C:\Program Files\HitachiSoft\MasterPlex QT 2.0\
Browse...
InstallShield
< Back
Next >
Cancel

Figure 2.2 Install Shield Wizard, Choose Destination Location window

3. To accept the default destination folder, click **Next**.

   To specify a different destination folder, click **Browse**, choose the folder, and click **Next**.

   ⇒ The Start Copying Files window appears (Figure 2.3).

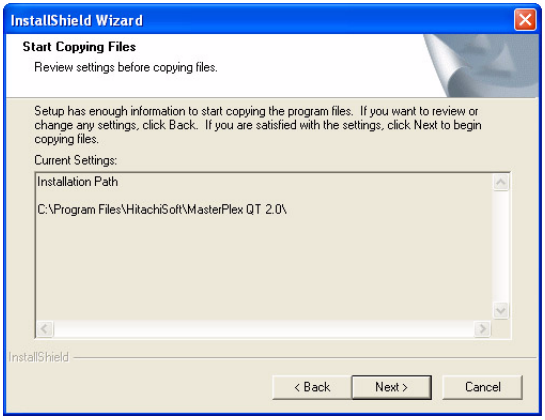

InstallShield Wizard
Start Copying Files
Review settings before copying files.
Setup has enough information to start copying the program files. If you want to review or change any settings, click Back. If you are satisfied with the settings, click Next to begin copying files.
Current Settings:
Installation Path
C:\Program Files\HitachiSoft\MasterPlex QT 2.0\
InstallShield
< Back
Next >
Cancel

Figure 2.3 InstallShield Wizard, Start Copying Files window

4. To copy the files to the selected directory, click **Next**.
   ⇒ After the installation is completed, the InstallShield Wizard Complete window appears (Figure 2.4).

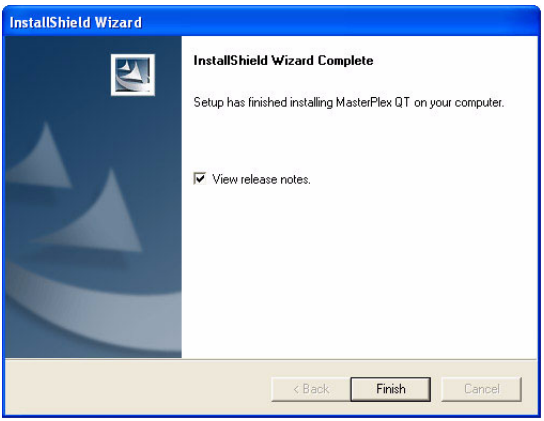


Figure 2.4 InstallShield Wizard Complete window

5. Choose the **View release notes** option, and click **Finish**.

2.3 Installing a License

1. Double-click the MasterPlex™ QT icon on the workstation desktop.
   ⇒ The License Information dialog box appears (Figure 2.5).

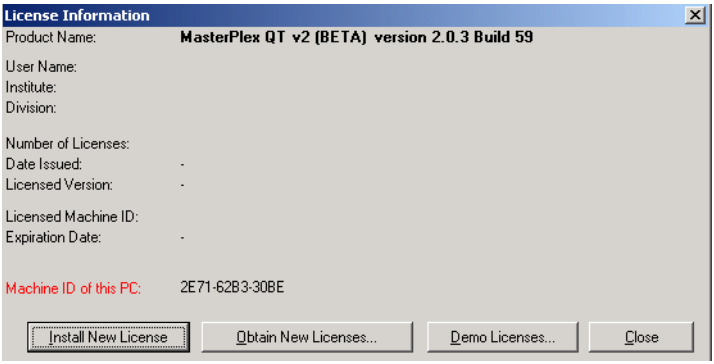


Figure 2.5 License Information dialog box

2. To view instructions on how to obtain a license (*.lic), click **Obtain New Licenses**.
3. After you have obtained a license, click **Install New License**.
   ⇒ The Open dialog box appears.
4. Use the Open dialog box to locate the license (*.lic) and double-click the file.
   ⇒ The license is installed.

CHAPTER 3

GETTING STARTED

This chapter provides a brief overview of data analysis using Masterplex™ QT 2.0. It also explains how to start the software, import a Luminex™ 100 results file (.csv), and the user interface components.

3.1 Overview of MasterPlex™ QT Analysis

MasterPlex QT software analyzes results files (.csv) from the Luminex 100 system. The analysis steps include:

- Import a Luminex results file (.csv)
- Designate well types (standard, unknown, background, or control) and well groups (identifies members of a standard data set or replicate unknowns)
- Define the standard data set (enter standard concentrations and select a model equation for the standard curve)
- Associate or *link* a standard data set to an unknown group(s)
- Compute the analyte concentrations
- Save the Luminex results file in MasterPlex QT file format (.mlx). The .mlx file includes information associated with the file (for example, well definitions and interpolated concentrations)

After the concentrations are calculated, you can:

- view the results in graphs or several different report formats
- create a *virtual plate* (a simulated microtiter plate) that contains data from user-selected actual plates (.csv or .mlx)
- generate a Dose-Reponse curve and determine the Log EC50 value for user-selected data in a virtual plate

3.2
Starting MasterPlex™ QT

- On the desk top, double-click the MasterPlex QT icon . Alternatively, click the Windows start menu button and select **Programs > MasterPlex QT 2.0 > MasterPlex QT 2.0**.

  ⇒ The MasterPlex QT user interface appears and displays the Plate Wizard and Navigator window (Figure 3.1).

  For more information about the Plate Navigator window, see page 3.14.

NOTE: The Plate Wizard appears if the **Enable plate wizard at start up** option is chosen in the Application preferences or the **Display wizard at startup** option is chosen in the Plate Wizard.

The Plate Wizard guides you through the steps to import a Luminex™ results file (.csv) or create a virtual plate. For more information on virtual plates, see Chapter 6 on page 6.1.

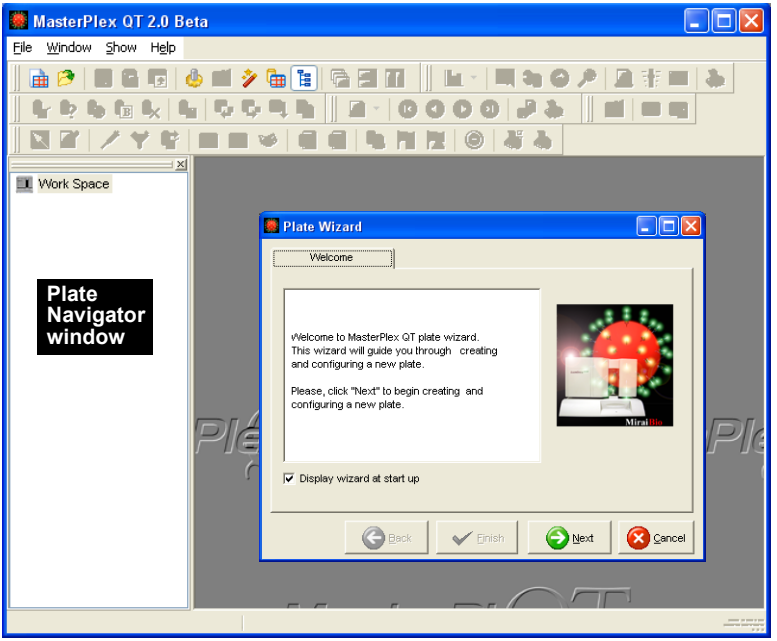


Figure 3.1 MasterPlex™ QT user interface
At start up, the Plate Wizard and Navigator window are displayed

3.3
Importing Luminex™ Results

To begin a MasterPlex QT analysis, import a .csv file from the Luminex 100 system using the Plate Wizard, toolbar, or menu bar commands.

NOTE: The Luminex default directory is named Output.

Importing Luminex Results Using the Plate Wizard

1. If the Plate Wizard is not open, click the **Plate Wizard** button .
   ⇒ The Plate Wizard appears (Figure 3.1).
2. In the Welcome tab of the Plate Wizard, click **Next**.
   ⇒ The Select Plate Type tab appears (Figure 3.2).

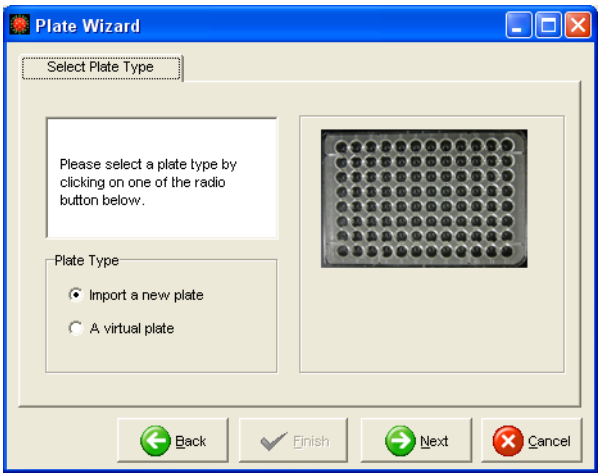


Figure 3.2 Plate Wizard
Select Plate Type tab

3. Choose the **Import a new plate** option and click **Next**.
   ⇒ The Import File tab appears (Figure 3.3).

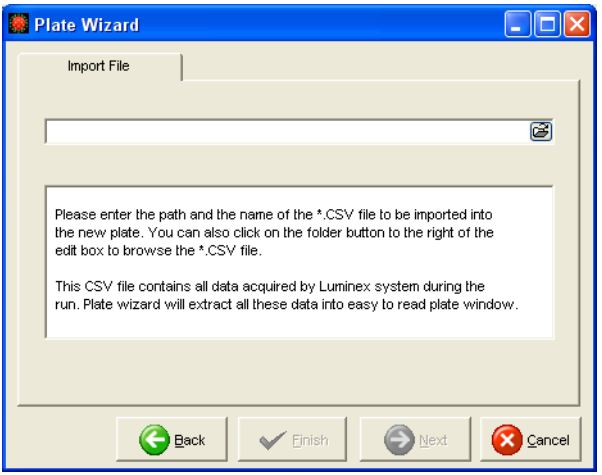


Plate Wizard
Import File
Please enter the path and the name of the *.CSV file to be imported into the new plate. You can also click on the folder button to the right of the edit box to browse the *.CSV file.
This CSV file contains all data acquired by Luminex system during the run. Plate wizard will extract all these data into easy to read plate window.
Back
Finish
Next
Cancel

Figure 3.3 Plate Wizard
Import File tab

4. Enter the file path for the .csv that you want to import. Alternatively, click the **Browse** button .
   ⇒ The Open dialog box appears (Figure 3.4).

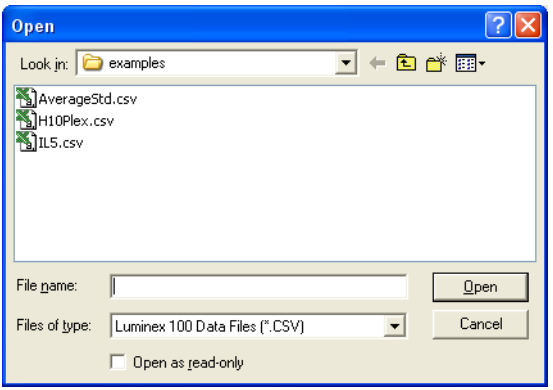


Figure 3.4 Open dialog box

5. Navigate to the directory of the .csv that you want to import.
6. Select one or more .csv files and click **Open**.

   To select adjacent files, press and hold the **Shift** key while you click the first and last file in the selection. To select nonadjacent files, press and hold the **Ctrl** key while you click the files of interest.

7. Click **Finish** in the Plate Wizard.
   ⇒ The Plate window opens and displays the results data (Figure 3.5).

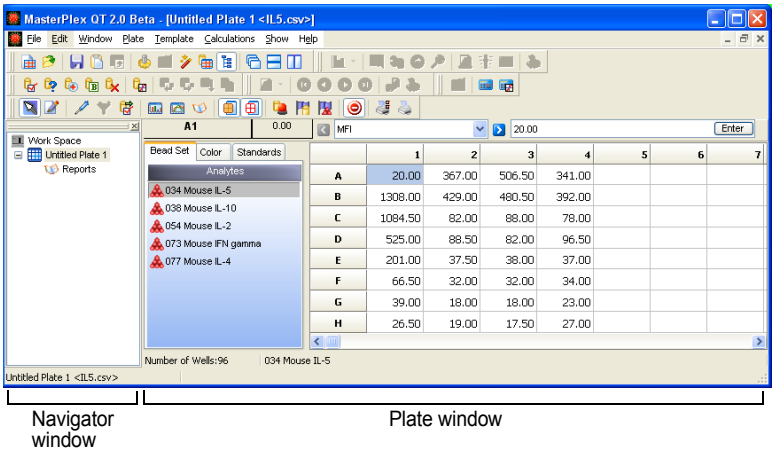


Figure 3.5 Plate window displaying results data
More than one Plate window can be open at the same time.

8. To import additional Luminex results files using the Plate Wizard, click the Plate Wizard button and repeat step 1 to step 6. Each set of results data is displayed in a separate Plate window.

Importing Luminex Results Using the Toolbar or Menu Bar

You can import a Luminex results file using the toolbar or menu bar.

1. To import a .csv file, click the **New** button or select **File** > **New Plate** from the menu bar.
   ⇒ The Open dialog box appears (Figure 3.6).

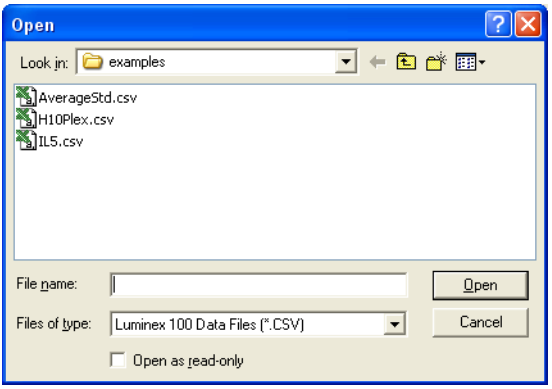


Figure 3.6 Open dialog box

2. Navigate to the directory of the .csv that you want to import.

NOTE: The Luminex default directory is named Output.

3. Select one or more .csv files and click **Open**.

   To select adjacent files, press and hold the **Shift** key while you click the first and last file in the selection. To select nonadjacent files, press and hold the **Ctrl** key while you click the files of interest.

   ⇒ The Plate window opens and displays the results data (Figure 3.7).

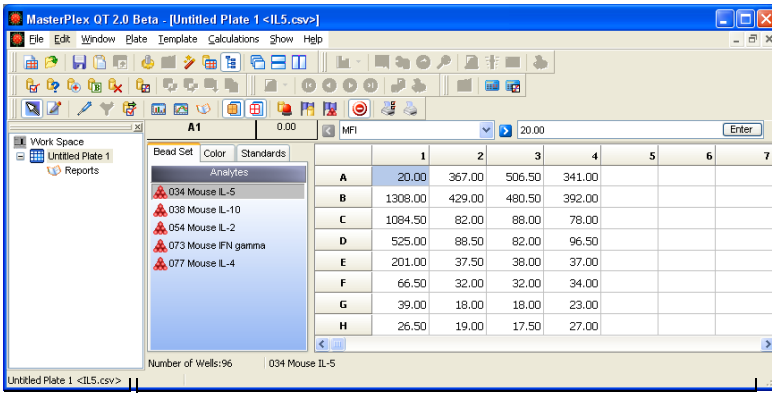


Figure 3.7 Navigator window, Plate window
More than one Plate window can be open at the same time.

4. To import additional Luminex results files, repeat step 1 to step 3. Each set of results data is displayed in a separate Plate window.

3.4
Using Windows Explorer to Import .csv or Open .mlx Files

1. Open Windows Explorer and adjust the window size so that you can view both the MasterPlex™ QT and Windows® Explorer application windows.
2. Use Windows Explorer to navigate to the .csv or .mlx file(s) that you want to open.
3. Select the file(s) of interest, then click and hold the mouse button while you drag the selected file(s) to the MasterPlex QT application window (Figure 3.8).

   To select adjacent files, press and hold the **Shift** key while you click the first and last file in the selection. To select nonadjacent files, press and hold the **Ctrl** key while you click the files of interest.
4. Release the mouse button.

   ⇒ The file(s) open in MasterPlex QT.

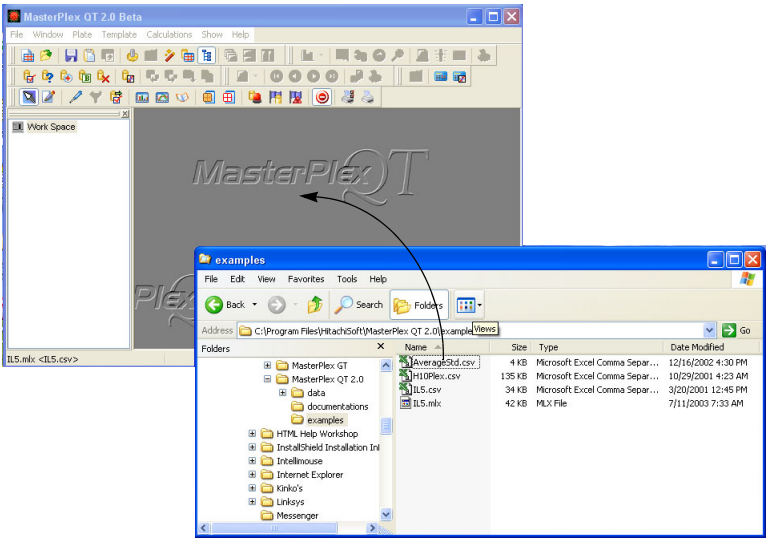


Figure 3.8 MasterPlex QT and Windows® Explorer application windows
Use a drag-and-drop operation to open a .csv or .mlx file(s) in the MasterPlex QT application window

3.5
Viewing Data in the Plate Window

1. If more than one Plate window is open, click the **Cascade** , **Tile Horizontally** , or **Tile Vertically** button to arrange the Plate windows for easier viewing.
2. To change the data displayed in the well grid:
   a. Click an analyte in the Bead Set panel.
   b. Make a selection from the data type drop-down list.
      ⇒ The well grid displays the data for the selected analyte.

   Figure 3.9 shows the components of the Plate window. Table 3.1 lists the types of data available for display in the well grid.
3. To view background-subtracted data, click the **Subtract background** button .
   ⇒ The Plate window displays background-subtracted data.

   For more information on background calculation options, see *Background Type* on page A.4.)

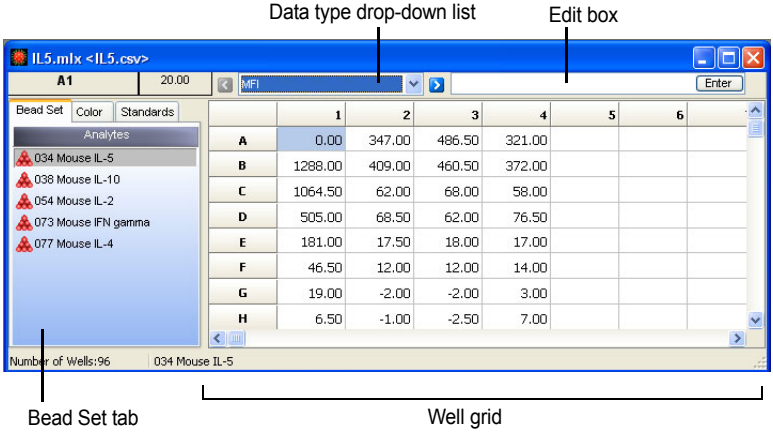


Figure 3.9 Plate window and Bead Set tab
Bead Set tab shows the analytes in the plate.

Plate Window Components

Well grid	A representation of a microtiter plate that displays the well contents for the analyte selected from the Bead Set panel and data type selected from the data drop-down list.
Data type drop-down list	Shows the types of data available for display in the well grid. Make a selection from this drop-down list to choose the data type displayed in the well grid. Click the drop-down arrow to view the list and select a data type. Alternatively, click the arrows to scroll forward or backward through the list. (See Table 3.1 for a description of the data types.)
Edit box	Displays the selected data type value for the active (selected) well. Some data types can be edited (see Table 3.1). Enter a new value in this box to edit well data.
Bead set tab	Displays a list of the analytes (bead sets) in an assay.
Color tab	Shows the color that represents each analyte in the multi-well chart. To change a color for an analyte, right-click the color swatch, and choose or define a color in the color palette that appears.
Standard tab	Displays a list of the local or imported standard curves for the plate.

Table 3.1 Data Types in the well grid

Data Type	Description	Edit Data
Median Fluorescence Intensity (MFI)	The median fluorescence intensity measured by the Luminex™ 100 system for a bead set count.	No
Count	The number of beads (per bead set) detected by the Luminex™ 100 system (specified by the user in the Luminex software).	No
Concentration	The analyte concentration that is computed (interpolated or extrapolated) from the user-selected standard curve.	Yes
Dilution	The dilution factor for the well.	Yes
Standard/ Independent Value	Standard Value: Analyte concentration for a standard. Independent Value: The agent concentration associated with a well that is a member of a regression data set. A Dose-Reponse curve is generated from a regression data set.	Yes Yes
Sample Names	User-specified name for the well.	Yes
Active Well	A check mark indicates the well is active and the well data are included in the calculation of concentrations or a Dose-Reponse curve.	Yes
Well Colors	The color that represents each analyte in the bar chart.	Yes
Group Numbers	The group number of the well. Wells that belong to the same group have the same group number.	No
Standard Links	Shows the standard number that is linked to each well or well group.	No

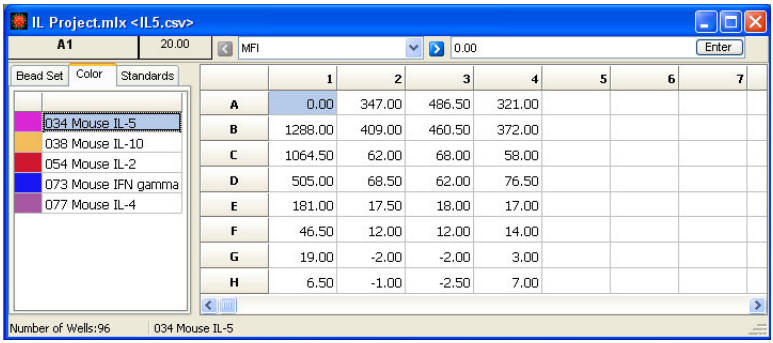


Figure 3.10 Plate window and Color tab

The Color tab shows the color that represents each analyte in the multi-well chart. To change a color for an analyte, right-click the color swatch, and choose or define a color in the color palette that appears.

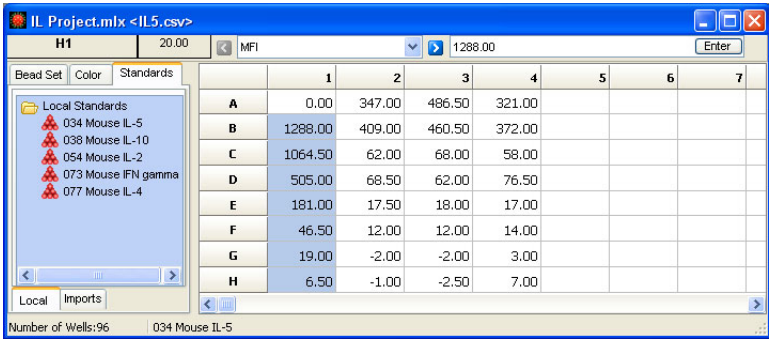


Figure 3.11 Plate window and Standards tab

Click a tab at the bottom of the Standards tab to display a list of the local or imported standards for the plate. For more information about standards, see Chapter 5 on page 5.1.

3.6
Thresholds

You can enter an MFI, count, or concentration threshold for a plate. The software can identify wells that contain data less than the user-specified threshold.

To set a threshold(s):

1. Click the **Preferences** button .
   ⇒ The Preferences dialog box appears (Figure 3.12).

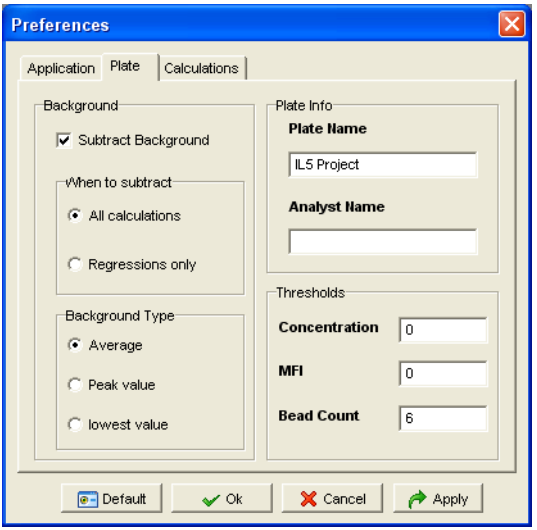


Figure 3.12 Preferences dialog box, Plate tab

2. Click the Plate tab.
3. Enter the concentration, MFI, or bead count threshold.
4. Click **Apply** when you are finished.
5. To return the plate preferences to the factory set defaults, click **Default**.

To identify wells in the Plate window that contain data less than threshold:

1. Make a selection from the Bead Set panel (Figure 3.13).
2. Select the data type (MFI, count, or concentration) from the data-type drop-down list.

3. To identify wells with MFI data less than threshold, click the button.

   ⇒ A red border is placed around the well (Figure 3.13).

   To identify wells with bead count data less than threshold, click the button.

   ⇒ A red border is placed around the well.

   To identify wells with concentrations less than threshold, click the button.

   ⇒ A red border is placed around the well.

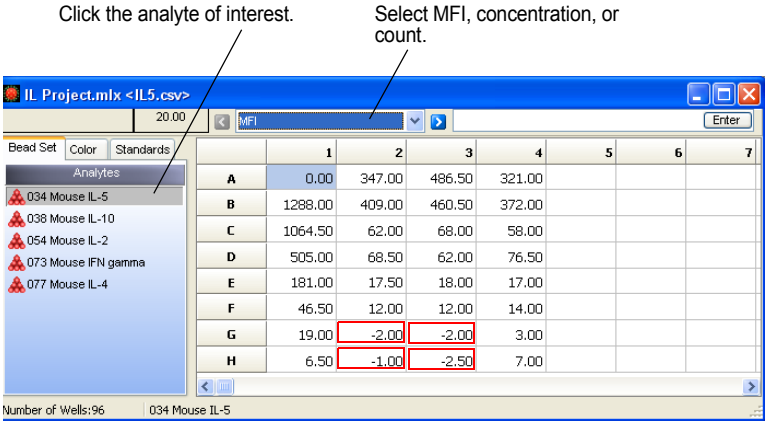


Figure 3.13 Well grid in the Plate window
Red border identifies wells with a MFI value less than threshold for the selected analyte.

NOTE: The threshold buttons (, , or) are mutually exclusive. Only one is active at a time.

3.7
The Plate Navigator

At startup, MasterPlex™ QT displays the Plate Navigator (Figure 3.14). The plate navigator workspace shows a tree of the windows that are open during a session.

The Plate Navigator organizes the open windows that are associated with a plate. It provides a convenient way to change the view in the main display area.

- To view a particular window, click the item in the Plate Navigator window (Figure 3.15).
- To show or hide the Plate Navigator, click the button.

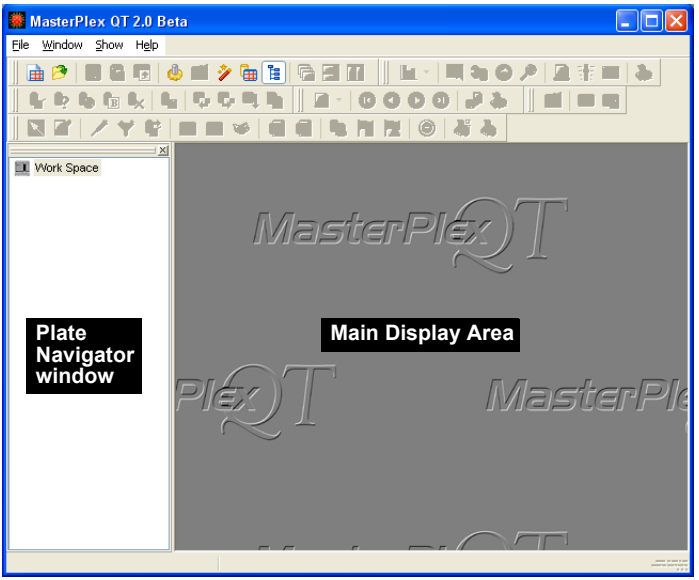


Figure 3.14 Plate Navigator window at start up
If no windows are open in the main display area, the Plate Navigator window is empty.

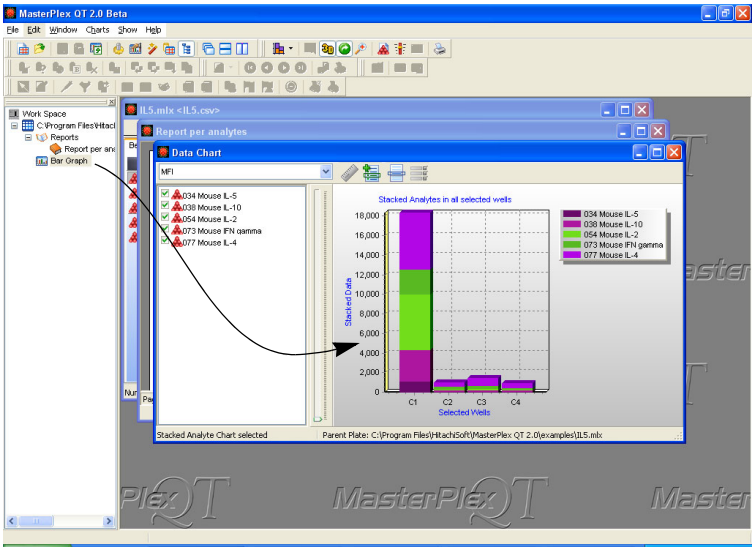


Figure 3.15 Plate Navigator window

If multiple windows are open, click an item in the Plate Navigator to view it in the main display area.

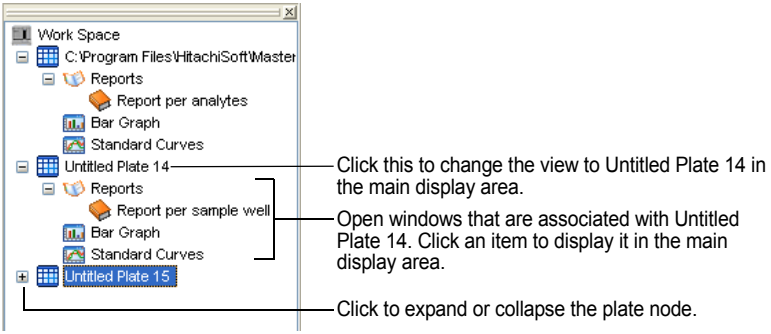

Work Space
C:\Program Files\HitachiSoft\Master
Reports
Report per analytes
Bar Graph
Standard Curves
Untitled Plate 14
Reports
Report per sample well
Bar Graph
Standard Curves
Untitled Plate 15
Click this to change the view to Untitled Plate 14 in the main display area.
Open windows that are associated with Untitled Plate 14. Click an item to display it in the main display area.
Click to expand or collapse the plate node.

Figure 3.16 Plate Navigator

Shows the windows that are open in the main display area.

Table 3.2 shows the types of windows available in the software.

Table 3.2 MasterPlex™ QT windows

Window Name	Displays...	See...
Plate	a .csv or .mlx file	Figure 3.17
Standard Curves	the Standard Curves window for the current Plate window	Figure 3.18
Data Chart	the current Data Chart window for the selected plate	Figure 3.19
Report	a report that was generated for the current plate	Figure 3.20

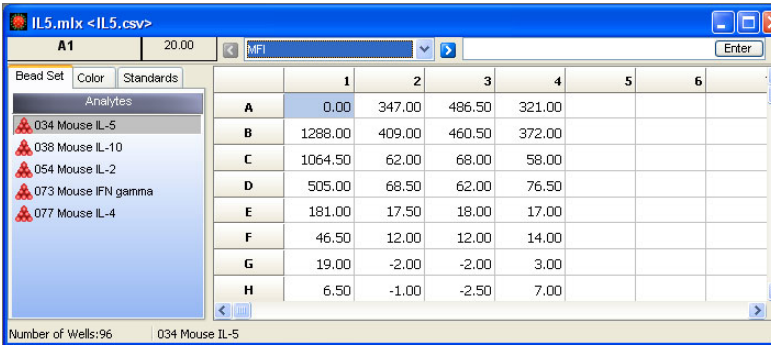


Figure 3.17 Plate window

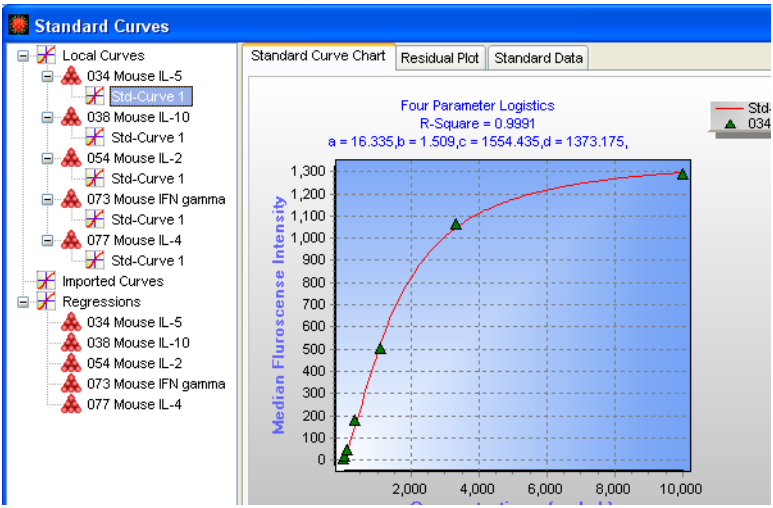


Figure 3.18 Standard Curves window

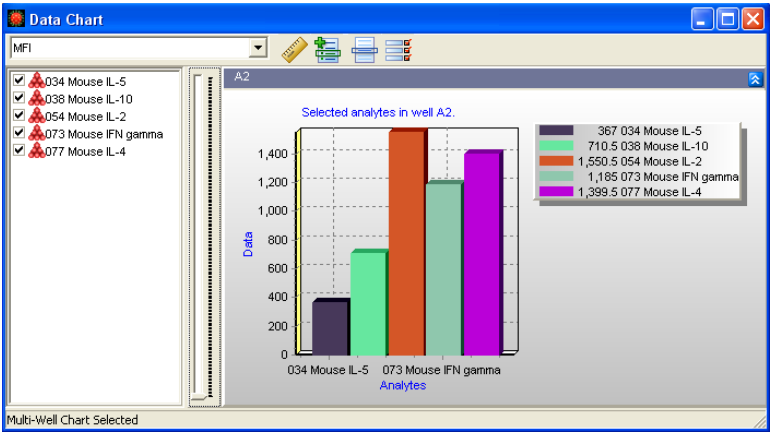
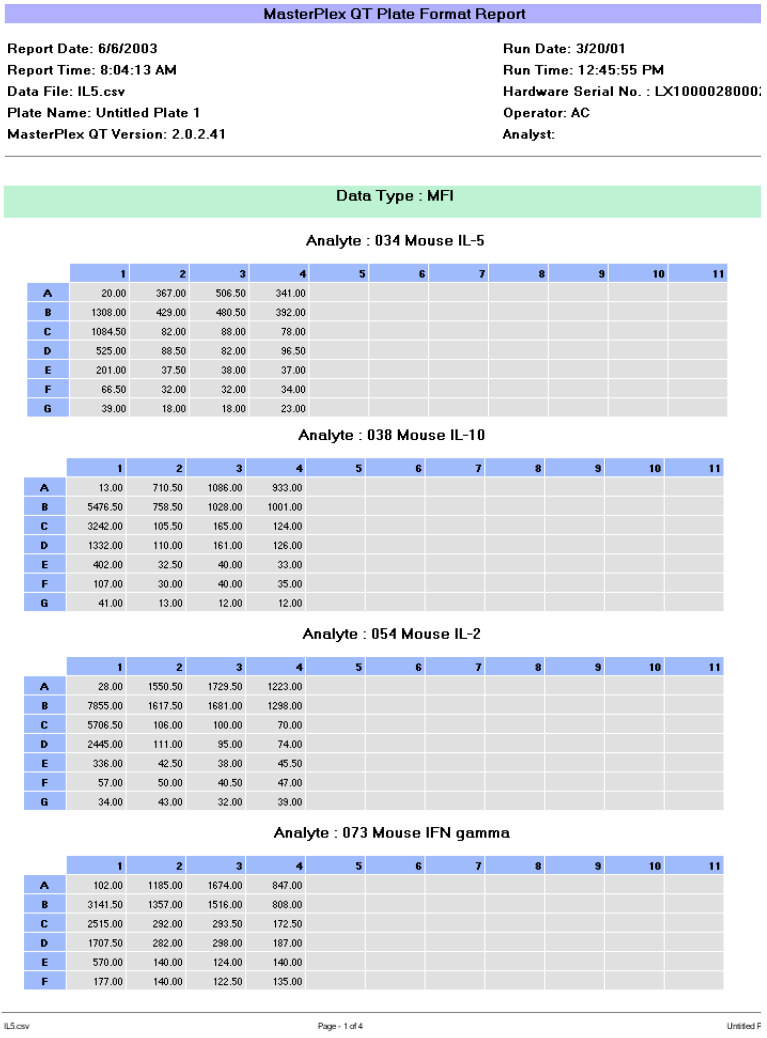


Figure 3.19 Data Chart window



MasterPlex QT Plate Format Report

Report Date: 6/6/2003
Report Time: 8:04:13 AM
Data File: IL5.csv
Plate Name: Untitled Plate 1
MasterPlex QT Version: 2.0.2.41

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX1000028000
Operator: AC
Analyst:

Data Type : MFI

Analyte : 034 Mouse IL-5

	1	2	3	4	5	6	7	8	9	10	11
A	20.00	367.00	506.50	341.00							
B	1308.00	429.00	480.50	392.00							
C	1084.50	82.00	88.00	78.00							
D	525.00	88.50	82.00	96.50							
E	201.00	37.50	38.00	37.00							
F	66.50	32.00	32.00	34.00							
G	39.00	18.00	18.00	23.00							

Analyte : 038 Mouse IL-10

	1	2	3	4	5	6	7	8	9	10	11
A	13.00	710.50	1086.00	933.00							
B	5476.50	758.50	1028.00	1001.00							
C	3242.00	105.50	165.00	124.00							
D	1332.00	110.00	161.00	126.00							
E	402.00	32.50	40.00	33.00							
F	107.00	30.00	40.00	35.00							
G	41.00	13.00	12.00	12.00							

Analyte : 054 Mouse IL-2

	1	2	3	4	5	6	7	8	9	10	11
A	28.00	1550.50	1729.50	1223.00							
B	7855.00	1617.50	1681.00	1298.00							
C	5706.50	106.00	100.00	70.00							
D	2445.00	111.00	95.00	74.00							
E	336.00	42.50	38.00	45.50							
F	57.00	50.00	40.50	47.00							
G	34.00	43.00	32.00	39.00							

Analyte : 073 Mouse IFN gamma

	1	2	3	4	5	6	7	8	9	10	11
A	102.00	1185.00	1674.00	847.00							
B	3141.50	1357.00	1516.00	808.00							
C	2515.00	292.00	293.50	172.50							
D	1707.50	282.00	298.00	187.00							
E	570.00	140.00	124.00	140.00							
F	177.00	140.00	122.50	135.00							

IL5.csv Page - 1 of 4 Untitled F

Figure 3.20 Report viewer, Plate format report

3.8
Saving Plate Data

After you import a Luminex results file (.csv), the data can be saved to a MasterPlex™ QT file format (.mlx). The .mlx file includes all data associated with a plate such as well definitions and computed (interpolated or extrapolated) concentrations.

To save results data (.csv) to a MasterPlex file (.mlx):

1. Click the **Save** button . Alternatively, select **File** > **Save** from the main menu.
   ⇒ The Save As dialog box appears (Figure 3.21).

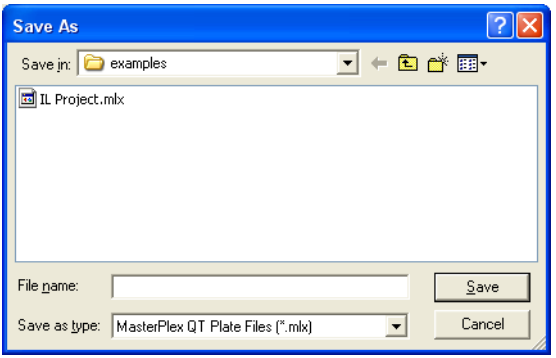


Figure 3.21 Save As dialog box

2. Confirm the default directory where the file will be saved or choose another directory.
3. Enter a file name and click **Save**.

Opening a MasterPlex File (.mlx)

1. Click the **Open** button . Alternatively, select **File** > **Open Plate** from the main menu.
   ⇒ The Open dialog box appears (Figure 3.21).

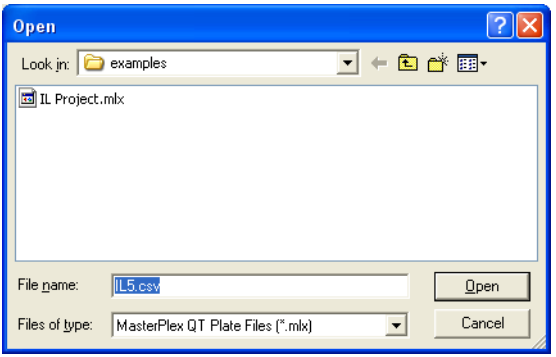


Figure 3.22 Save As dialog box

2. Confirm the default directory or choose another directory.
3. Select a file name (.mlx) and click **Open**.
   ⇒ A Plate window opens and displays the results data (Figure 3.23).

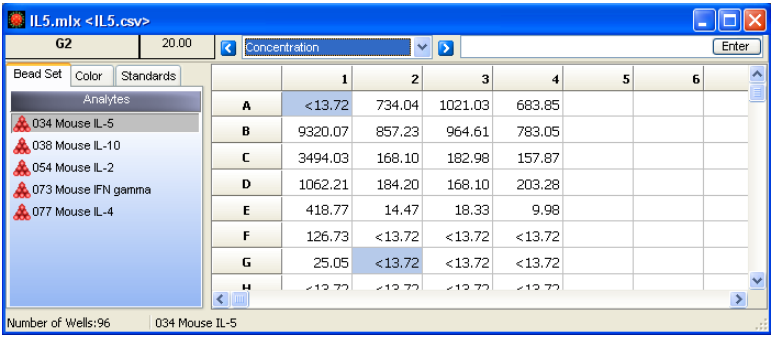


Figure 3.23 Plate window

CHAPTER 4

DEFINING A PLATE

After you import a Luminex results file (.csv), your analysis begins by defining a plate. This chapter explains how to define and save a plate. The steps to define a plate include:

- **Designate well type** to identify the standard, unknown, background, and control wells.
- **Designate well groups** to identify replicate unknowns, members of a standard data set, or members of a regression data set.
- **Create a standard data set(s)** by entering the concentration for each well in the standard data set and selecting a model equation for the standard curve. A plate can have more than one standard data set.
- **Link each well group to a standard data set** to specify the standard that is used to compute (interpolate or extrapolate) the analyte concentrations.

The plate definition can be saved as a template that can be applied to other plates. The Template Manager helps you manage your templates. For more information on templates, see *Working With Templates* on page 4.9.

4.1 Designating Well Type and Group

Selecting Wells

To select a well in the Plate window, click the well in the well grid. There are three ways to select multiple wells:

- To select adjacent wells (Figure 4.1), press and hold the mouse key while you drag the mouse cursor over the wells that you want to select. Click and release the mouse button to select the highlighted wells.
- To select adjacent wells, press and hold the **Shift** key while you click the first and last well in the selection.
- To select nonadjacent wells (Figure 4.2), press and hold the **Ctrl** while you click the wells.

	1	2	3	4
A	0.00	347.00	486.50	321.00
B	1288.00	409.00	460.50	372.00
C	1064.50	62.00	68.00	58.00
D	505.00	68.50	62.00	76.50
E	181.00	17.50	18.00	17.00
F	46.50	12.00	12.00	14.00
G	19.00	-2.00	-2.00	3.00
H	6.50	-1.00	-2.50	7.00

Figure 4.1 Well grid

*To select adjacent wells, press and hold the **Shift** key while you click the first and last well in the selection. Alternatively, press and hold the mouse key while you drag the mouse over the wells of interest.*

	1	2	3	4
A	0.00	347.00	486.50	321.00
B	1288.00	409.00	460.50	372.00
C	1064.50	62.00	68.00	58.00
D	505.00	68.50	62.00	76.50
E	181.00	17.50	18.00	17.00
F	46.50	12.00	12.00	14.00
G	19.00	-2.00	-2.00	3.00
H	6.50	-1.00	-2.50	7.00

Figure 4.2 Well grid

*To select nonadjacent wells, press and hold the **Ctrl** key while you click the wells of interest.*

Designating Well Type

Table 4.1 shows the types of wells that are available.

1. Select the well(s) that you want to define.
2. To define (or *mark*) the well(s), right-click the selection and choose **Mark Wells** and a well type from the pop-up menu that appears Figure 4.3. You can also use toolbar buttons or menu bar commands to define the wells (Table 4.1).

   ⇒ The well type is applied to the selected well(s).

NOTE: If the **Automatic Well Grouping** option is selected in the Preferences dialog box, the wells are automatically grouped when you set the well type. For more information on this option, see *Application Tab* on page A.1.

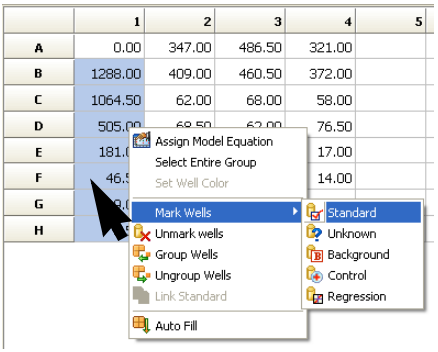


Figure 4.3 Well grid pop-up menu
Right click a well to display the pop-up menu

Table 4.1 Button and menu bar commands to define wells

Well Type	Button	Menu Bar Commands
Unknown Wells that contains analytes of unknown concentration.		Template > Mark Wells > Mark Unknown Well
Standard Wells that contains analyte of known concentration.		Template > Mark Wells > Mark Standard Well
Background Wells that contain no analytes.		Template > Mark Wells > Mark Background Wells
Control Wells that contain analytes that function as controls for a particular assay design.		Template > Mark Wells > Mark Control Wells
Regression Wells of a virtual plate that are members of a regression data set. (See *Generating a Dose-Reponse Curve* on page 6.11 for more information on regression analysis data sets.)		Template > Mark Wells > Mark Regression Wells

NOTE: To show or hide the color-coded well types, click the **Show well type** button .

3. To unmark a well, right-click the well and select **Un-Mark Wells** from the pop-up menu. Alternatively, select the well(s) and click the **Unmark wells** button or select **Template** > **Unmark well** from the menu bar.

If a well belongs to a group, unmarking the well also removes the well from the group.

4. Repeat step 1 and step 2 to mark and group other well(s).

Designating Well Groups

After you have defined the wells, the wells must be organized into *groups* so that the software can identify:

- Replicate unknowns
- A standard data set
- A regression analysis data set (for a virtual plate only) (For more information on virtual plates, see Chapter 6 on page 6.1.)

MasterPlex™ QT automatically places all background wells into one group. You can define one or more groups of control wells per plate.

NOTE: A group can include nonadjacent wells. A plate can have more than one group of standards or unknowns. A virtual plate can have more than one set of regression data. To show or hide the group borders, click the **Show well grouping** button .

NOTE: Grouping does not set replicate unknowns. Standard, unknowns, control and background groups can be treated as replicates to obtain statistics such as mean, standard deviation and CV% in the final report.

Automatic Well Grouping

If the **Automatic Well Grouping** option is chosen in the Preferences dialog box (Figure 4.4), the software automatically groups wells when you set the well type. The automatic well grouping is the factory set default.

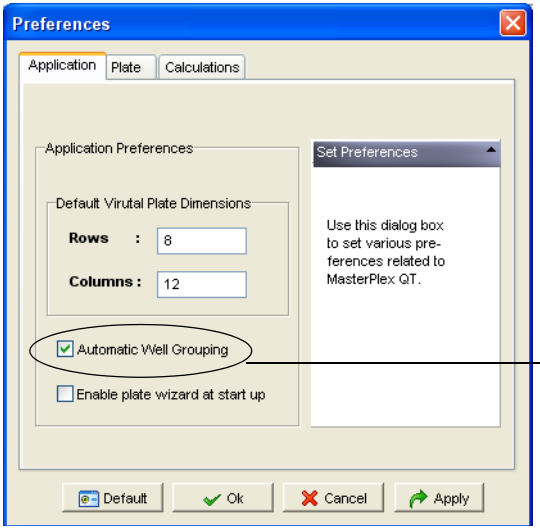


Figure 4.4 Preferences dialog box

*To display the Preference dialog box, click the button or select **File > Preferences** from the menu bar.*

Manually Grouping Wells

You can only place the same type of wells in a group.

1. Select the wells that you want to group.

NOTE: The grouping function is only available if you have selected wells that have been defined.

2. Right-click the selection, and select **Group Wells** from the pop-up menu that appears. Alternatively, click the selection, and click the **Group Wells** button or select **Template > Group Wells** from the menu bar.

   ⇒ A border appears around the grouped wells (Figure 4.5).
3. To display the well grouping (Figure 4.5), click a well.

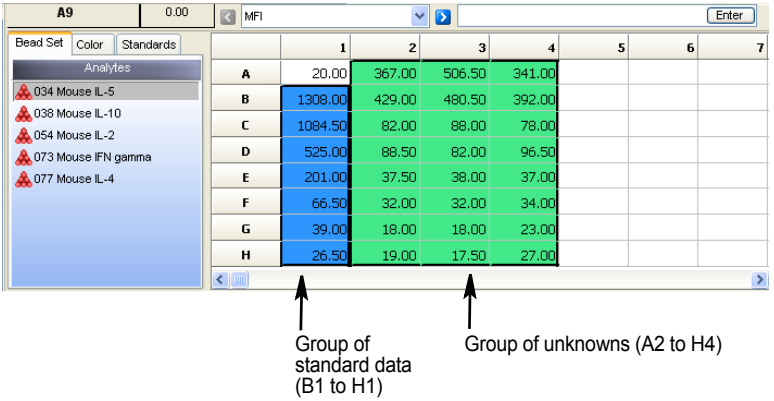


Figure 4.5 Well groups
A red border identifies a group in the well grid.

Ungrouping Wells

1. Right-click the group.

NOTE: The ungrouping function is only available if you have selected grouped wells.

2. Select **Ungroup wells** from the pop-up menu that appears. Alternatively, click the **Ungroup wells** button or select **Template** > **Ungroup wells** from the menu bar.
   ⇒ The grouping is removed.

Setting Standard Concentrations

After you define and group the standard wells, use the autofill feature to help you automatically enter the standard concentrations. For more information on specifying standard data, see Chapter 5 on page 5.1.

1. Right-click the standard data set and select **Auto Fill** from the popup menu. Alternatively, click the **Auto Fill** button or select **Template** > **Auto Fill** from the menu bar.
   ⇒ The Auto Fill dialog box appears (Figure 4.6).

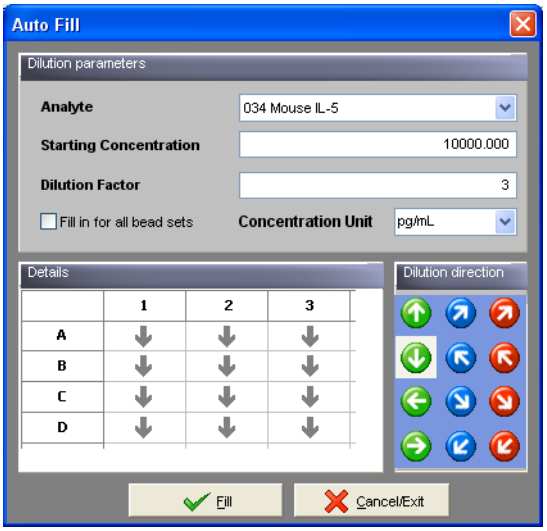


Figure 4.6 Auto Fill dialog box

2. Make a selection from the Analyte drop-down list.
3. Enter the starting concentration for the standard data set.
4. Enter the dilution factor.
5. Make a selection from the concentration unit drop-down list
6. To select a dilution direction for the standard data set, click a dilution direction arrow.

   ⇒ The gradient map shows the location and direction of the dilution gradient(s) (Figure 4.7).

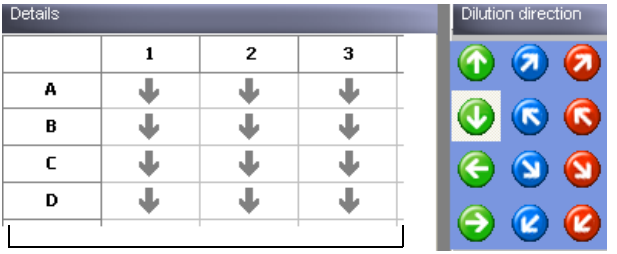

Details
1
2
3
A
B
C
D
Dilution direction
Click an arrow to choose a dilution direction.
This gradient map specifies a separate dilution gradient in each column of the standard data set. The starting concentration is at the top of a column.
Details
1
2
3
A
B
C
D
Dilution direction
This gradient map specifies one dilution gradient per standard data set. The starting concentration is at the upper left well and the end concentration is at the lower right well.

Figure 4.7 Example dilution gradient maps
Click a dilution direction arrow to choose the dilution gradient configuration for a standard data set

7. To specify the same starting concentration, dilution factor, and concentration units for all analytes in the standard data set, choose the **Fill in for all bead sets** option. To specify a different starting concentration, dilution factor, or concentration unit for a different analyte, repeat step 2 through step 4.
8. Click **Fill** when finished entering the concentration, dilution, and dilution direction for all analytes in the standard data set.

   ⇒ A confirmation message appears (Figure 4.8).

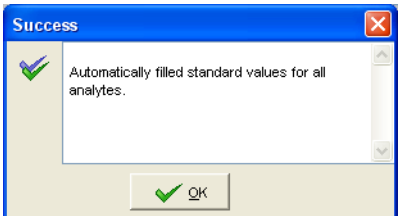


Figure 4.8 Message box confirming autofill

Linking a Standard Data Set

Background, control, and unknown wells must be associated with or *linked* to the standard data set that will be used to calculate concentrations. By default, the first standard that you define will be linked to the background, control, and unknown well groups.

If there is more than one standard on a plate, you can link a user-selected standard to a user-selected well group(s).

1. To link a well group to a standard data set, press and hold the **Ctrl** key while you click the group and the standard data set that you want to link.

NOTE: A standard data set can be linked to multiple groups of the same well type, but each group can have only one standard.

2. Right-click the selection and choose **Link Standard** from the pop-up menu that appears. Alternatively, click the **Link** button or select **Template > Link Standard** from the menu bar.

4.2
Working With Templates

A plate definition includes:

- well types and well groups
- standards (including standard concentrations, associated model equation, and concentration units)
- links between the standard(s) and well groups
- data calculated for the plate (for example, analyte concentrations, standard data curves, or regression analysis curves (for virtual plates only)

- data manually entered in the plate (for example, dilution factors or independent data values)
- plate preferences

You can save the plate definition as a template. You can apply a template to an active plate. Templates may also be exported, imported, or deleted.

NOTE: If a plate window is not open, you can still delete, import, or export templates; however, you cannot load, save, or overwrite a template.

Opening the Template Manager

The Template Manager is a tool that helps you manage your templates.

1. Click the **Template Manager** button . Alternatively select **Plate > Template Manager** from the menu bar.
   ⇒ The Template Manager appears (Figure 4.9).
2. Click a template in the Available Templates list to view information about the template.

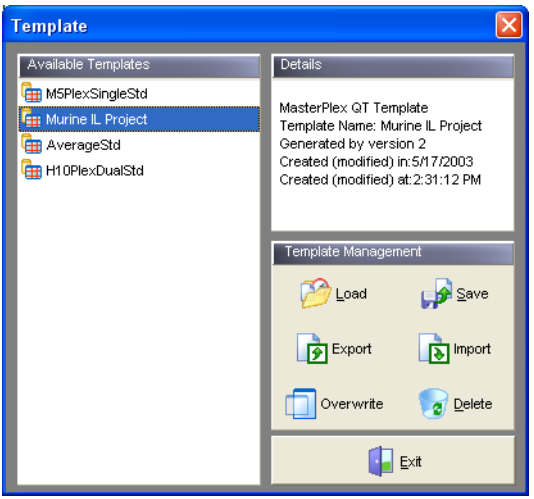


Figure 4.9 Template Manager shows available templates
Click a template to view information about the template.

Saving a Template

You can save the current plate definition to a template.

1. After you have finished defining a plate, open the Template Manager and click the **Save** button .
   ⇒ The Template Name box appears (Figure 4.10).

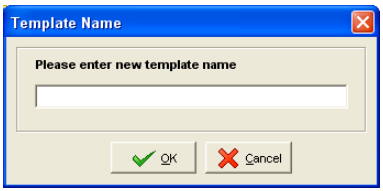


Figure 4.10 Template Name box

2. Enter a name for the template and click **OK**.
   ⇒ The new template is added to the Available Template list.

Loading a Template

You can apply or *load* a saved template to the current plate.

1. In the Template Manager, select the template that you want to apply to the plate.
2. Click the **Load** button .
   ⇒ The template is applied and the well grid shows the new well attributes (well type, well group, and links to standard data sets).

Overwriting a Template

You can overwrite an existing template with the current plate definition.

1. In the Template Manager, select the template that you want to overwrite
2. Click the **Overwrite** button .
   ⇒ A confirmation box appears (Figure 4.11).

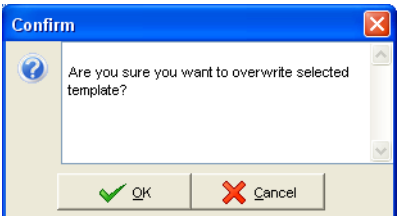


Figure 4.11 Confirmation box

3. Click **OK** to overwrite the selected template with the current plate definition.

Exporting a Template

You can export a template to a user-specified location.

1. In the Template Manager, click the template that you want to export.
2. Click the **Export** button .
   ⇒ The Save As dialog box appears (Figure 4.12).

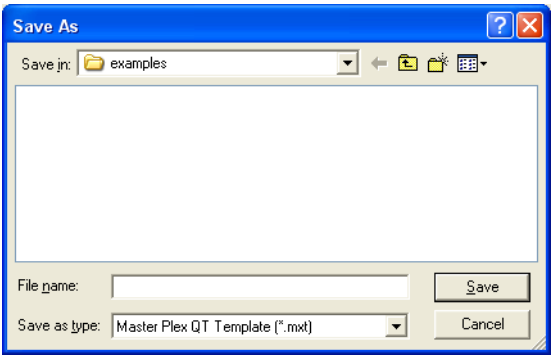


Figure 4.12 Save As dialog box

3. Choose the directory for the template that you want to export.
4. Enter a name for the template (*.mxt).

NOTE: A template must have a .mxt file extension. Changing the extension will render the exported template unusable.

Importing a Template

You can import a template (.mxt) from a user-specified location. Templates from MasterPlex QT 1.0 can also be imported.

1. In the Template Manager, click **Import** button .
   ⇒ The Open dialog box appears (Figure 4.13).

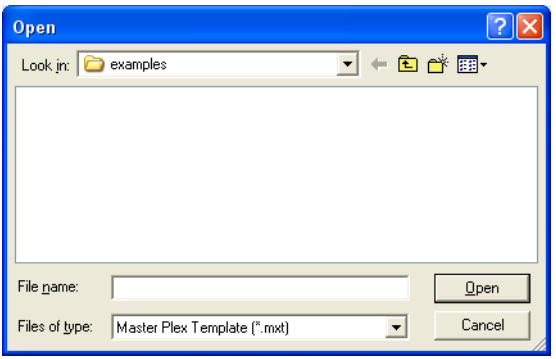


Figure 4.13 Open dialog box

2. Choose the directory with the template that you want to import.
3. Select the template and click **Open**.
   ⇒ The template name is added to the Template Manager.

Deleting a Template

You can delete a template (.mxt) from the system.

1. In the Template Manager, click the template that you want to delete.
2. Click **Delete** button .
   ⇒ A confirmation box appears (Figure 4.14).

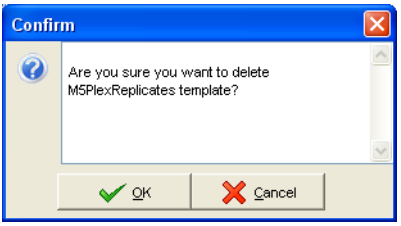


Figure 4.14 Confirmation box

3. Click **OK** to delete the template.
   ⇒ The template is removed from the Template Manager.

WARNING: This permanently removes the template from the system.

4.3
Setting Preferences

Preferences are user-modifiable software settings. They are displayed in the Preferences dialog box.

- To open the Preferences dialog box (Figure 4.15), click the **Preferences** button . Alternatively, select **File** > **Preferences** from the menu bar.

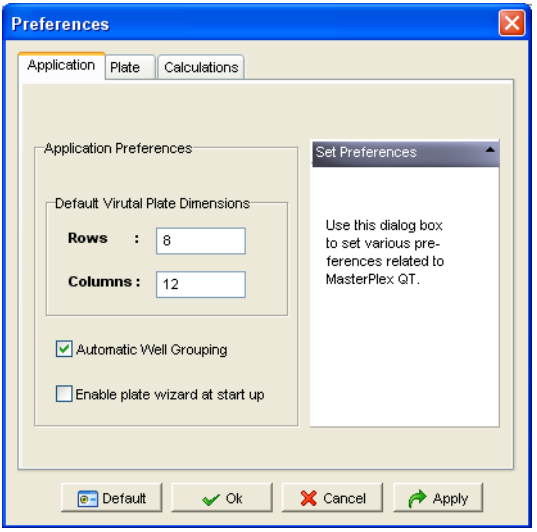


Figure 4.15 Preferences dialog box
Application tab default settings

Application Preferences

The application preferences (Figure 4.15) include:

Default Virtual Plate Dimensions	The default number of rows and columns for a virtual plate (displayed by the plate wizard during virtual plate setup).
Automatic Well Grouping	Choose this option to automatically group wells after they are defined.
Enable plate wizard at startup	Choose this option to display the plate wizard at startup.

1. To set the row and column dimensions of the well grid for a virtual plate displayed by the plate wizard, enter the number of rows and columns.
2. If you do not want to automatically group wells after they are defined, remove the check mark from the **Automatic Well Grouping** option.
3. If you do not want to display the plate wizard at program start up, remove the check mark from the **Enable plate wizard at start up** option.
4. Click **Apply** when you are finished.
5. To return the application preferences to the factory set defaults, click **Default**.

Plate Tab

The Plate preferences specify:

- how to compute background and when to subtract background
- threshold values for concentration, MFI, and bead count
- the plate and analyst name

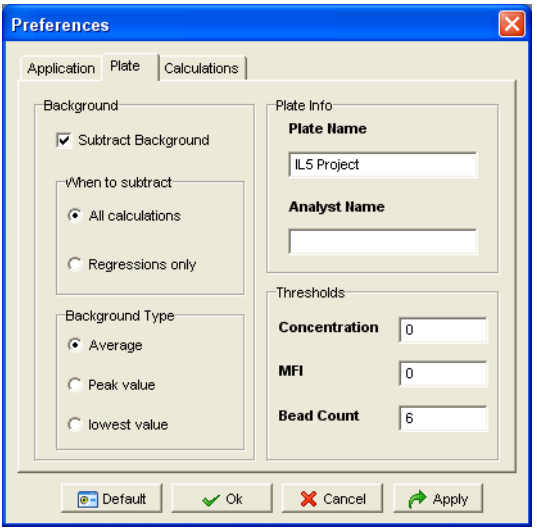


Figure 4.16 Preferences dialog box
Plate tab default settings

NOTE: If a plate (.csv or .mlx) is not open, the Preferences dialog box does not display the Plate tab.

Background Options

You can specify whether you want to consider background data in the calculation of analyte concentrations. There are two methods of computing background-subtracted analyte concentrations: All Calculations or Regressions Only.

Subtract Background	Choose this option if you want to compute background-subtracted analyte concentrations. If this option is not chosen, the background MFI value is not considered during calculation of the analyte concentrations.
All Calculations	This method of computing background-subtracted analyte concentrations subtracts the background MFI from each member of the standard data set, then fits the standard curve. The method subtracts background MFI from unknown MFI, then interpolates or extrapolates the unknown analyte concentration from the standard curve.
Regressions Only	This method of computing background-subtracted analyte concentrations subtracts the background MFI from each member of the standard data set, then fits the standard curve. The method does not subtract the background MFI from the unknown MFI before interpolating or extrapolating the unknown analyte concentration.

NOTE: The **All Calculations** method is recommended. The **Regressions Only** method provides backward compatibility with data generated in MasterPlex™ QT 1.0.

Background Type

If there are three or more background wells in the assay, choose one of the following methods for computing background MFI.

Average	Background (Bkg) MFI = (Bkg MFI_1 + Bkg MFI_2 +... Bkg MFI_n)/n where n = the number of background wells in the plate
Peak Value	Highest background MFI value.
Lowest Value	Lowest background MFI value.

Plate Info

Plate Name	Displays the name assigned to the result file in the Luminex™ 100 software. To edit the plate name, enter a new name.
Analyst Name	Displays the analyst name entered in the Luminex™ 100 software. To edit the analyst name, enter a new name.

Thresholds

You can enter an MFI, count, or concentration threshold for a plate. The software automatically marks wells that contain data less than the user-specified threshold with a red border (Figure 4.18).

To set a threshold(s):

1. Enter the MFI, count, or concentration threshold in the Preferences dialog box (Figure 4.17).

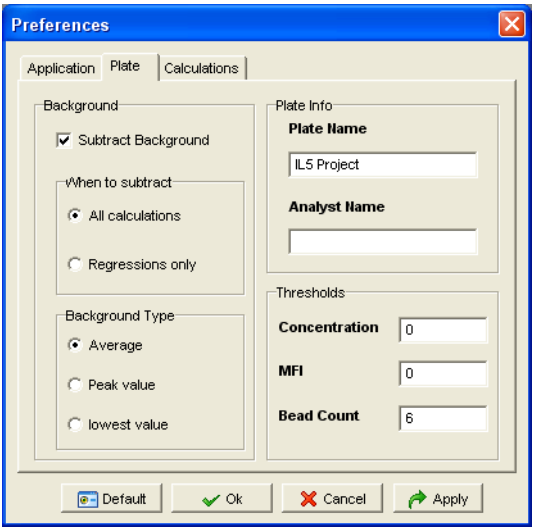


Figure 4.17 Preferences dialog box, Plate tab

2. Click **Apply** when you are finished.
3. To return the plate preferences to the factory set defaults, click **Default**.

To identify the wells that contain data less than threshold:

1. Choose an analyte in the Bead Set panel.
2. Select the data type (MFI, count, or concentration) from the data-type drop-down list.
3. To identify wells with:
   a. MFI less than threshold, click the button.
   b. Bead count data less than threshold, click the button.
   c. Concentration less than threshold, click the button.

   A red border marks wells that contain data less than threshold for the selected analyte (Figure 4.18).

NOTE: The threshold buttons (, , or) are mutually exclusive. Only one is active at a time.

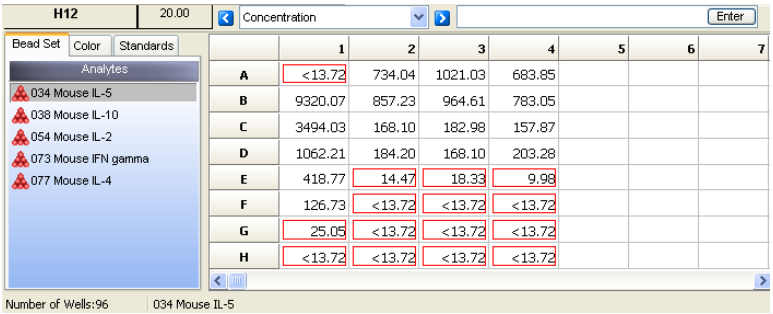


Figure 4.18 Well grid
Red border identifies wells with concentration less than user-specified threshold for the selected analyte

Calculations Preferences

The Calculations preferences (Figure 4.19) specify how to:

- fit the standard curve when there are replicate standard data sets
- display concentrations for unknowns that were diluted prior to the assay (the diluted concentration or the original undiluted concentration can be displayed)

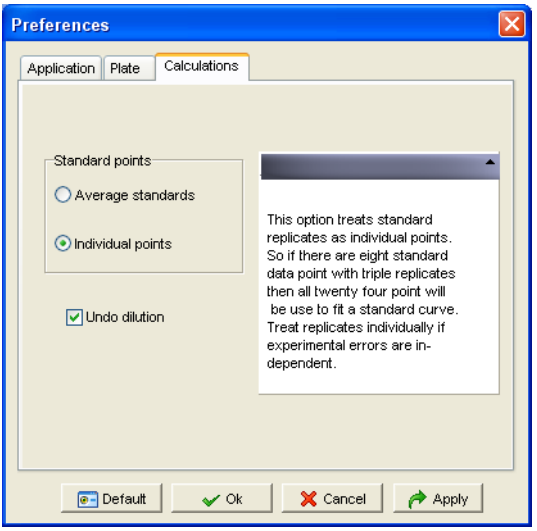

Preferences
Application
Plate
Calculations
Standard points
Average standards
Individual points
Undo dilution
This option treats standard replicates as individual points. So if there are eight standard data point with triple replicates then all twenty four point will be use to fit a standard curve. Treat replicates individually if experimental errors are in-dependent.
Default
Ok
Cancel
Apply

Figure 4.19 Preferences dialog box, Calculations tab

Standard Points

If a plate contains replicate standard data sets, there are two ways to fit a standard curve.

Average standards	The replicate standard data points are averaged and the standard curve is fitted to the single set of averaged data. Choose this option if the experimental errors are dependent on each another.
Individual points	The replicate standard data points are not averaged and the standard curve is fitted to all of the data points. For example, if there are three replicates of eight standard wells, the standard curve is fitted using all 24 data points. Choose this option if the experimental errors are independent of each other. This option is set by default. In most cases, if you're not sure about the nature of the distribution of the experimental errors, try this option first.

Dilution for Unknowns
Samples can be diluted prior to the assay and analysis. After MasterPlex QT interpolates the diluted unknown analyte concentrations from the standard curve, it can compute and display the original, undiluted concentration in the Plate window.

Original concentration = Diluted concentration * Dilution Factor

The dilutions factors are manually entered in the Plate window. (For more information see *Working With Diluted Unknowns* on page 5.8.

4. Click **Apply** when you are finished.

To return the plate preferences to the factory set defaults, click **Default**.

4.4
Saving a Plate

The software saves the imported results (.csv) in MasterPlex™ QT native file format (.mlx). The .mlx file also includes the:

- well and group definitions or the associated template
- well and bead set (analyte) color
- data calculated for the plate (for example, analyte concentrations, standard data curves, or regression analysis curves (for virtual plates only)
- data manually entered in the plate (for example, dilution factors or independent data values)
- plate preferences

1. To save a plate, click the **Save** button .
   ⇒ The Save As dialog box appears (Figure 4.20).

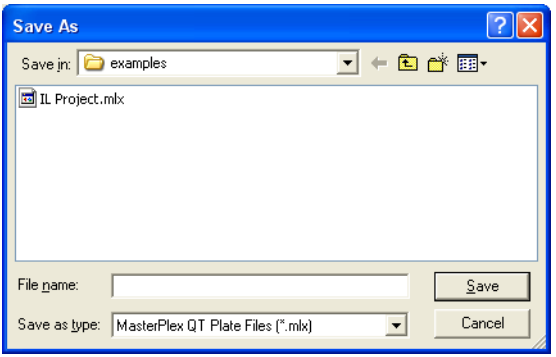


Figure 4.20 Save As dialog box
Save the current plate data in native .mlx format.

2. Enter a name for the file.
3. Click **Save**.

CHAPTER 5 STANDARD CURVES & ANALYTE CONCENTRATION

This chapter explains how to generate standard curves and compute (interpolate or extrapolate) analyte concentrations from the standard curves.

5.1 Specifying Standard Data

Each well in a standard data set represents an x,y data point. The MFI value is plotted on the y-axis and the concentration is plotted on the x-axis. MasterPlex™ QT uses regression analysis to fit a user-specified model equation to the standard data set and generate a standard curve.

NOTE: The standard curve may not pass through each point in the standard data set.

The software computes the R^2 value ($0 \leq R^2 \leq 1$) for the model equation. R^2 measures the goodness of fit of the model equation to the standard data set (where $R^2 = 1$ is the probability that the model predicts the data perfectly).

The steps to create a standard curve include:

1. Mark the standard wells.
2. Group the wells in a standard data set.
3. Link the standard data set to the unknown well group(s) of interest. (The analyte concentrations are interpolated from the standard curve that is linked to the unknown well group.)
4. Enter the standard concentrations.
5. Select a model equation for the standard data set.
6. Calculate the standard curves.

NOTE: A plate can have more than one standard data set. The standard data sets may have different concentrations or model equations.

Entering Standard Concentrations

Enter the standard concentrations after you mark the standard wells, group them into a standard data set, and link the standard data set to a group(s) of unknown wells. (For more information on setting well types and groups, see *Designating Well Type and Group* on page 4.1.)

There are two ways to enter standard concentrations:

- Use the autofill feature to automatically enter the analyte concentrations
- Manually enter the analyte concentrations

Using the Autofill Feature

The autofill feature enters the analyte concentrations for selected standard wells based on the user-specified starting concentration and dilution factor.

1. Open the results of interest (.csv or .mlx) and select the standard data set (Figure 5.1).

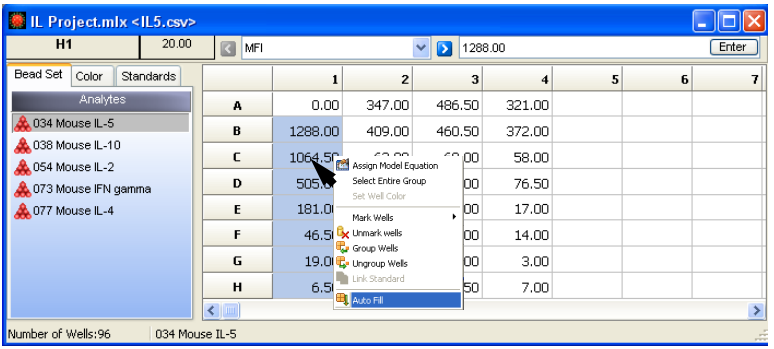


Figure 5.1 Standard wells selected in the well grid

2. Right-click a standard well and select **Auto Fill** from the pop-up menu that appears. Alternatively, click the **Auto Fill** button .
   ⇒ The Auto Fill dialog box appears (Figure 5.2).

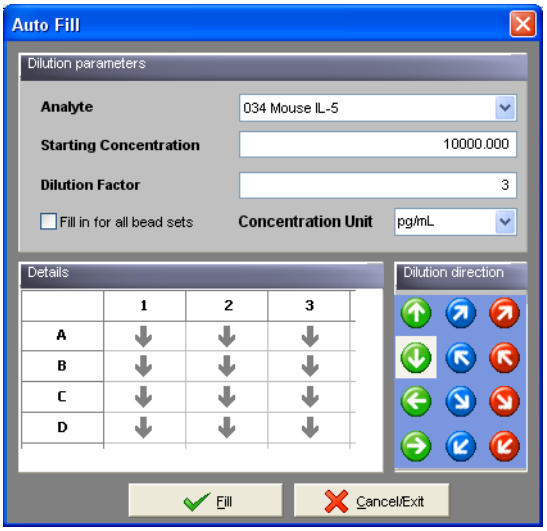
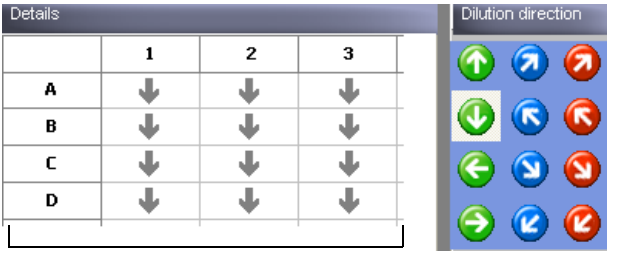


Figure 5.2 Auto Fill dialog box

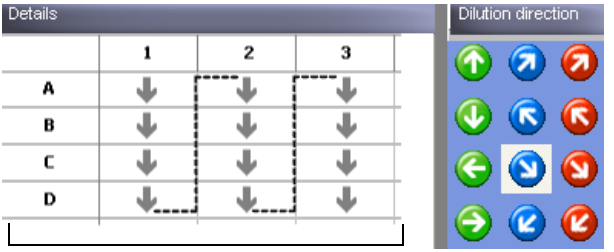
3. Make a selection from the Analyte drop-down list.
4. Enter the starting concentration for the standard data set.
5. Enter the dilution factor.
6. Make a selection from the concentration unit drop-down list
7. To select a dilution direction for the standard data set, click a dilution direction arrow.

   ⇒ The gradient map shows the location and direction of the dilution gradient(s) (Figure 5.3).



Click an arrow to choose a dilution direction.

This gradient map specifies a separate dilution gradient in each column of the standard data set. The starting concentration is at the top of a column.



This gradient map specifies one dilution gradient per standard data set. The starting concentration is at the upper left well and the end concentration is at the lower right well.

Figure 5.3 Example dilution gradient maps
Click a dilution direction arrow to choose the dilution gradient configuration for a standard data set

8. If you want to specify the same starting concentration, dilution factor, and concentration units for all analytes in the standard data set, choose the **Fill in for all bead sets** option. If you want to specify a different starting concentration, dilution factor, or concentration unit for a different analyte, repeat step 2 through step 4.
9. Click **Fill** when finished entering the concentration, dilution, and dilution direction for all analytes in the standard data set.

   ⇒ A confirmation message appears (Figure 5.4).

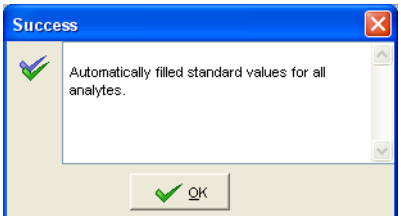


Figure 5.4 Message box confirming autofill

10. Select Standard/Independent Values from the data type drop-down list to view the analyte concentrations for the selected standards (Figure 5.5).

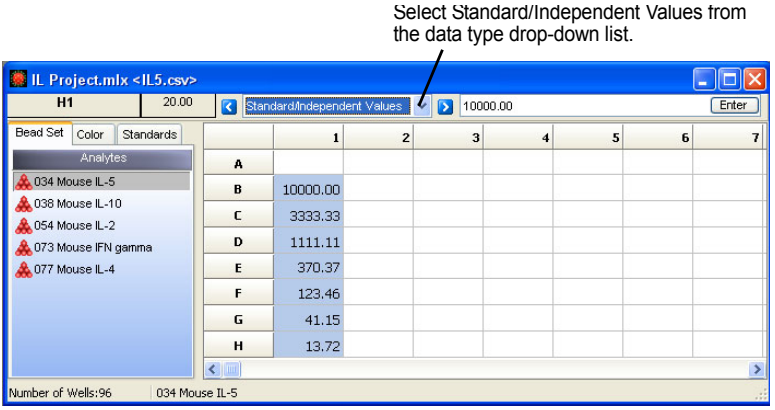


Figure 5.5 Standard concentrations for the selected analyte

The autofill feature entered the analyte concentrations for the selected standard set based on a user-specified starting concentration of 10,000 and a dilution factor of 3.

Entering Standard Concentrations Manually

1. Open the results of interest (.csv or .mlx).
2. Click the **Well Editor** button . Alternatively, select **Plate** > **Edit Wells** from the menu bar.
   ⇒ The Plate window is in edit mode.

NOTE: You can only edit the standard concentrations when the plate window is in edit mode.

3. Select an analyte from the Bead Set panel (Figure 5.6).

4. Select **Standard/Independent Values** from the data type drop-down list.
5. In the well grid, click the well that you want to edit.
   ⇒ The concentration value for the selected well is displayed.
6. Enter a concentration value and click **Enter**.
   ⇒ The well grid displays the new concentration value.
7. To enter other standard concentration values for the same analyte, repeat step 4 and step 5.
8. To enter standard concentration values for a different analyte, repeat step 2 through step 5.

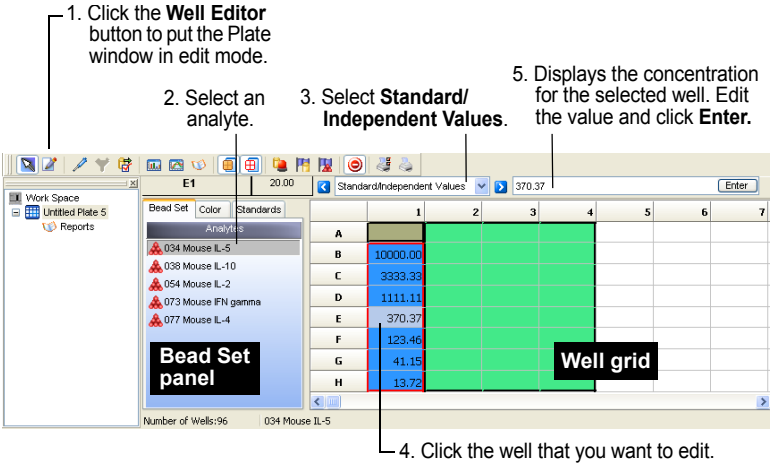


Figure 5.6 Plate window
Steps to manually enter a standard concentration

Selecting a Model Equation for the Standard Data Set

1. Select an analyte from the Bead Set panel (Figure 5.6).
2. In the well grid, select a standard data set.
3. Right-click the standard data set and select **Assign Model Equation** from the pop-up menu that appears. Alternatively, click the **Select Model** button or select **Calculations** > **Model Equations** from the menu bar.
   ⇒ The Model Equations dialog box appears (Figure 5.7).

NOTE: The Model Equations dialog box only appears if you selected a standard data set.

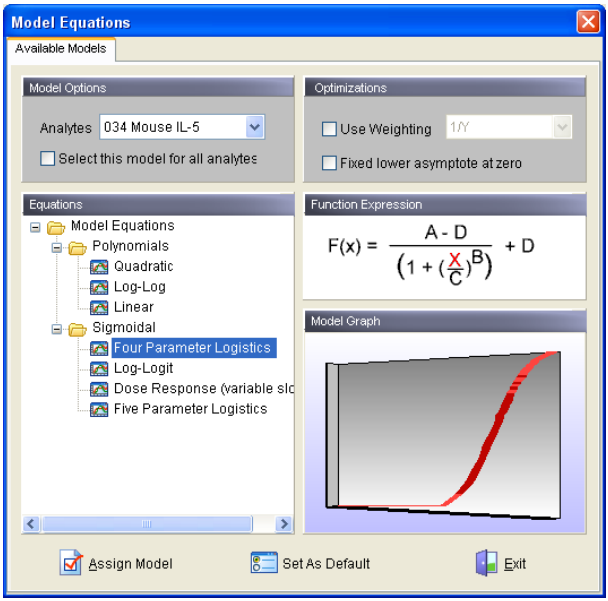


Figure 5.7 Model Equations dialog box
Model equations available for regression analysis of a standard data set

4. Select a model equation.
5. To apply the selected model to all analytes, choose the **Select this model for all analytes** option.
6. To fix the bottom asymptote of the four parameter logistics curve or the five parameter logistics curve to zero (sets A = 0), choose the **Fix bottom at zero** option.
7. To apply weighting during curve fitting, choose the **Use Weighting** option and select a weighting method from the drop-down list.
8. Click **Assign Model**.
9. To choose a model equation for another analyte, repeat step 1 to step 3, and click **Assign Model**.

NOTE: For more information about model equations and weighting methods, see Appendix C.

5.2
Working With Diluted Unknowns

If you need to dilute a sample prior to an assay, you can specify a dilution factor in the well grid. MasterPlex™ QT can compute the diluted or original analyte concentration.

For a diluted unknown:

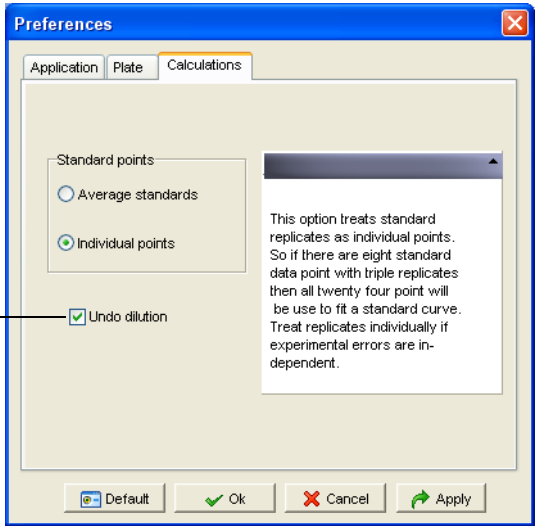
Original concentration = Dilution factor * Calculated concentration.

Setting the Concentration Calculation Preference

When you work with diluted unknowns, you must specify whether the software computes the diluted or original analyte concentration.

1. Click the **Preferences** button . Alternatively, select **File > Preferences** from the menu bar.
   ⇒ The Preferences dialog box appears (Figure 5.8).
2. Click the Calculations tab.
3. To display the diluted analyte concentration for a diluted unknown in Plate window, confirm that the **Undo dilution** option is not chosen.
4. To display the original, undiluted analyte concentration for a diluted unknown in the Plate window, choose the **Undo dilution** option.

NOTE: If you change the Undo dilution option, you must recalculate the analyte concentrations.


Preferences
Application
Plate
Calculations
Standard points
Average standards
Individual points
This option treats standard replicates as individual points. So if there are eight standard data point with triple replicates then all twenty four point will be use to fit a standard curve. Treat replicates individually if experimental errors are in-dependent.
Undo dilution
Default
Ok
Cancel
Apply

Choose this option to display the original, undiluted analyte concentration in the well grid.

Figure 5.8 Preferences dialog box, Calculations tab

Editing a Dilution Factor

1. Click the **Well Editor** button . Alternatively, select **Plate** > **Edit Wells** from the menu bar.
   ⇒ The Plate window is in edit mode.

NOTE: You can only edit a dilution factor when the Plate window is in edit mode.

2. Select **Dilution** from the data type drop-down list.
3. In the well grid, click the dilution factor(s) that you want to edit. To select all of the dilution factors for a group, right-click a dilution factor and choose **Select Entire Group** from the pop-up menu that appears.
4. To edit a single selected dilution factor, use the arrows. Alternatively, double-click the dilution factor in the well, edit the value in the edit box, and click **Enter**.
5. To edit the dilution factor for several wells simultaneously:
   a. Click the **Selection Tool** button to put the plate in selection mode.

b. Click the wells that you want to edit,

c. Click the edit box and enter the dilution factor.

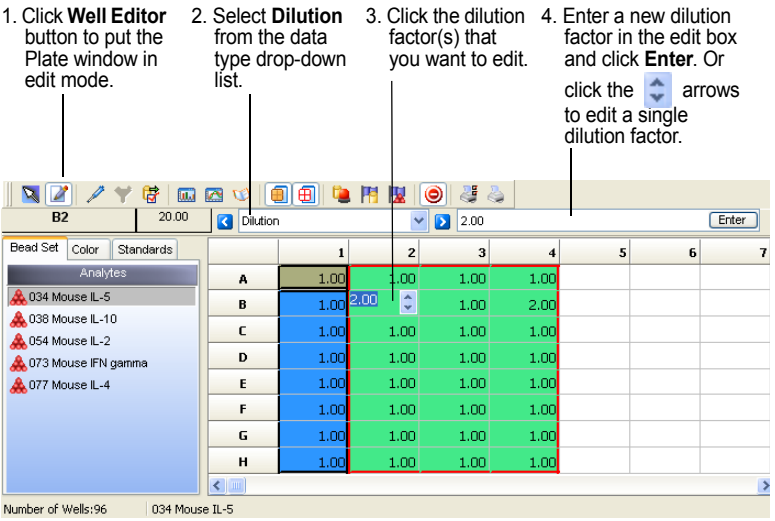

1. Click **Well Editor** button to put the Plate window in edit mode.
2. Select **Dilution** from the data type drop-down list.
3. Click the dilution factor(s) that you want to edit.
4. Enter a new dilution factor in the edit box and click **Enter**. Or click the arrows to edit a single dilution factor.
B2
20.00
Dilution
2.00
Enter
Bead Set
Color
Standards
Analytes
034 Mouse IL-5
038 Mouse IL-10
054 Mouse IL-2
073 Mouse IFN gamma
077 Mouse IL-4
1 2 3 4 5 6 7
A 1.00 1.00 1.00 1.00
B 1.00 2.00 1.00 2.00
C 1.00 1.00 1.00 1.00
D 1.00 1.00 1.00 1.00
E 1.00 1.00 1.00 1.00
F 1.00 1.00 1.00 1.00
G 1.00 1.00 1.00 1.00
H 1.00 1.00 1.00 1.00
Number of Wells:96
034 Mouse IL-5

Figure 5.9 Steps to edit a dilution factor

5.3
Generating Standard Curves & Computing Analyte Concentrations

MasterPlex™ QT carries out a two step calculation sequence when it fits the standard curves. The software:

- fits a standard curve for all defined standard data sets
- interpolates or extrapolates analyte concentrations for the unknown groups that are linked to the standard data set

Standard Curve Preferences

If a standard data set includes replicates, MasterPlex™ QT can fit a standard curve two different ways:

Individual points method (default)	Treats each point in the standard data set individually for curve fitting. For example, if there are three replicates of eight standard wells, the software fits a standard curve using all 24 data points. If the experimental errors are independent, choose this option.
Average standards method	Computes the average of the replicate standard data points and fits the standard curve to the averaged points. If the experiment errors are dependent on each another, choose this option.

1. To view or change the standard curve preference, click the **Preferences** button . Alternatively, select **File** > **Preferences** from the menu bar.
   ⇒ The Preferences dialog box appears (Figure 5.10).

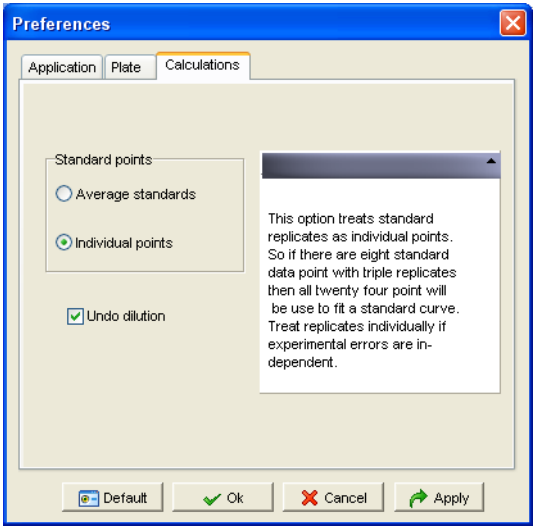

Preferences
Application
Plate
Calculations
Standard points
Average standards
Individual points
Undo dilution
This option treats standard replicates as individual points. So if there are eight standard data point with triple replicates then all twenty four point will be use to fit a standard curve. Treat replicates individually if experimental errors are independent.
Default
Ok
Cancel
Apply

Figure 5.10 Preferences dialog box, Calculations tab

2. Click the Calculations tab.

3. Choose a **Standard points** option, click **Apply**, and click **OK**.
4. To return all preference settings to the factory set defaults, click **Default**, and click **OK**.

Generating Standard Curves & Viewing Analyte Concentrations

1. To generate the standard curves and compute (interpolate or extrapolate) the analyte concentrations, click the **Calculate** button . Alternatively, select **Calculations** > **Calculate STD Curves** from the menu bar.

   ⇒ A message box confirms the calculations are completed (Figure 5.11).

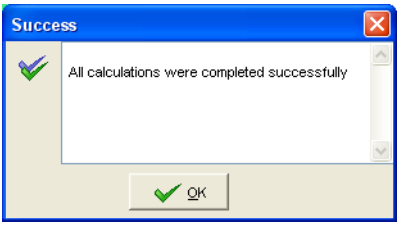


Figure 5.11 Message box

2. To view analyte concentrations in the Plate window, select **Concentration** from the data type drop-down list, and select an analyte in the Bead Set tab (Figure 5.12).

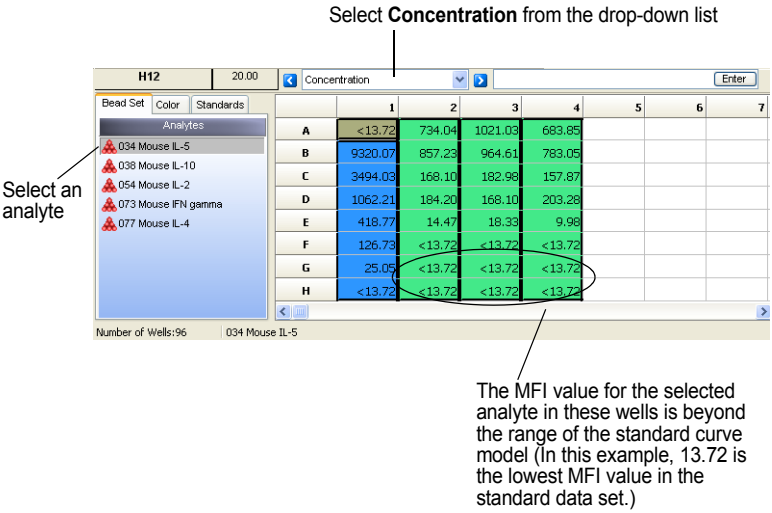

Select **Concentration** from the drop-down list
H12
20.00
Concentration
Enter
Bead Set
Color
Standards
Analytes
034 Mouse IL-5
038 Mouse IL-10
054 Mouse IL-2
073 Mouse IFN gamma
077 Mouse IL-4
Select an analyte
1 2 3 4 5 6 7
A <13.72 734.04 1021.03 683.85
B 9320.07 857.23 964.61 783.05
C 3494.03 168.10 182.98 157.87
D 1062.21 184.20 168.10 203.28
E 418.77 14.47 18.33 9.98
F 126.73 <13.72 <13.72 <13.72
G 25.05 <13.72 <13.72 <13.72
H <13.72 <13.72 <13.72 <13.72
Number of Wells:96
034 Mouse IL-5
The MFI value for the selected analyte in these wells is beyond the range of the standard curve model (In this example, 13.72 is the lowest MFI value in the standard data set.)

Figure 5.12 Well grid displays concentration data for the selected analyte

If the standard curve uses a sigmoidal model (for example, the four parameter logistics equation, Figure 5.13), the software interpolates the analyte concentration when:

Highest standard MFI ≤ MFI ≤ Lowest standard data point

The software extrapolates the analyte concentration when:

A < MFI < Lowest standard MFI

or

Highest standard MFI < MFI < D

where A is the bottom asymptote and D is the top asymptote of the sigmoidal curve (Figure 5.13).

A MFI value less than A or greater than D is beyond the range of the standard curve model and the concentration value cannot be extrapolated. If MFI < A, the well grid displays the lowest standard MFI preceded by < (Figure 5.12). If MFI > D, the well grid displays the highest standard MFI preceded by >.

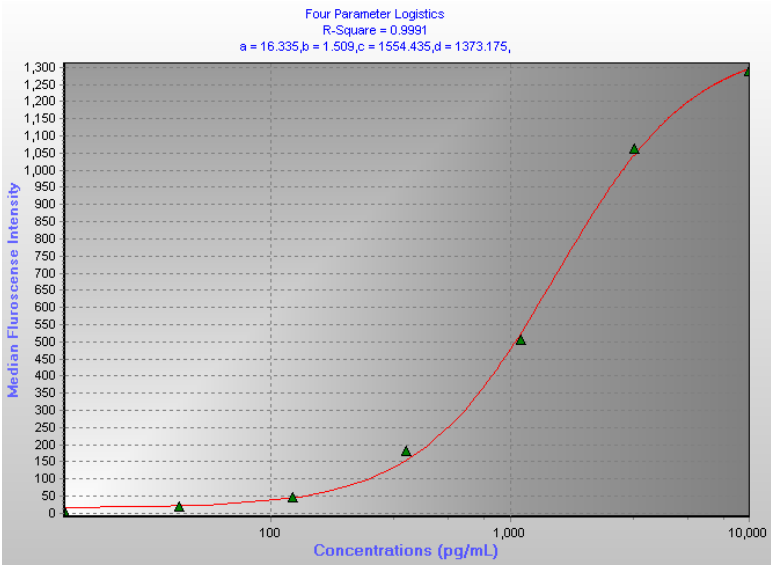


Figure 5.13 Four parameter logistics model equation, x-axis log scale
A = 16.335 (bottom asymptote), D = 1373.175 (top asymptote). MFI values less than A or greater than D are beyond the range of the model equation. Right-click the graph to select log scale for the x-axis.

5.4
Printing the Well Grid

You can print the contents of the well grid.

1. To set print preferences, click the **Plate Print Settings** button .
   ⇒ The Print Settings dialog box appears (Figure 5.14).

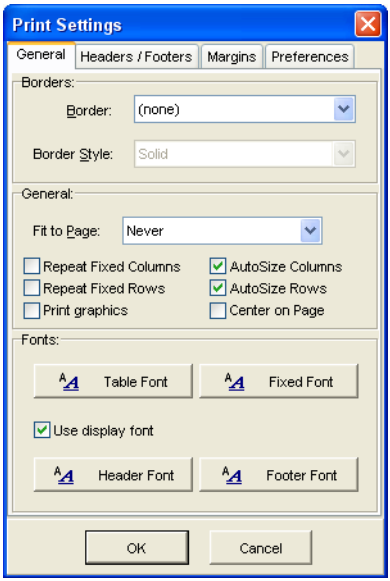


Figure 5.14 Print Settings dialog box

2. To display a print preview of the well grid, click the **Print** button .

   ⇒ The Print Preview dialog box displays the currently selected well contents (data type and analyte).

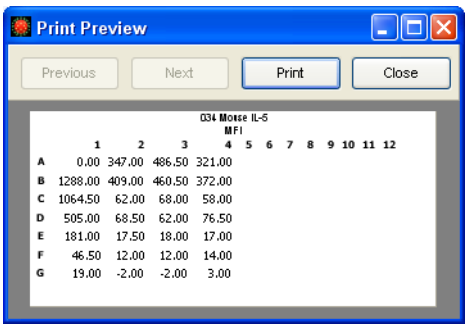


Figure 5.15 Print Preview dialog box
Preview the well grid contents.

3. To print the well grid, click **Print**.

5.5
Working With Standard Curves

After the standard curves are generated, you can view the curves, residual plots, the standard data, and information about the standard data in the Standard Curves window (Figure 5.16).

Opening the Standard Curves Window

1. Click the **Open Standard Curve** button . Alternatively, select **Plate > View Curves** from the menu bar.

   ⇒ The Standard Curves window opens and displays the Standard Curve Chart tab (Figure 5.16).

   The window displays a tree of local standard curves, imported standard curves, and regression curves for the active plate.

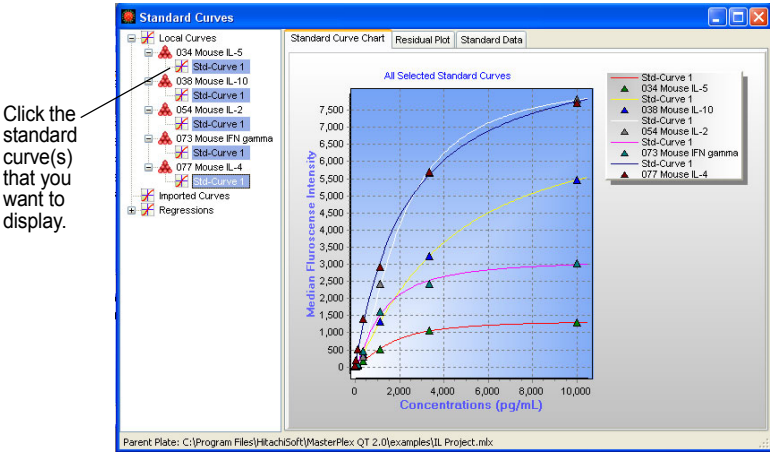


Figure 5.16 Standard curves window, Standard Curve Chart tab

2. To display a standard curve, click the curve(s) of interest in the data tree. To select multiple curves, press and hold the **Ctrl** key while you the click the curves.

NOTE: If the Standard Curves window shows data points only, you need to generate the standard curves (click the **Calculate** button).

Viewing the Residual Plot

The residual plot helps you identify outliers and detect non-constant variability in a standard curve. In a residual plot, each point represents the difference between the expected standard concentration and the calculated standard concentration for a selected analyte.

> Residual = Observed (or calculated) concentration - Expected concentration

The observed (calculated) concentration is interpolated or extrapolated from the standard curve. The expected concentration is the standard data value that you enter using the autofill function (or manually). If the residual is positive, we have detected a concentration that is greater than the expected value. If the residual is negative, the detected concentration is less than the expected value.

1. To view the residual plot, click the Residual Plot tab in the Standard Curves window.
2. Click the standard curve of interest.
   ⇒ The residual plot is displayed (Figure 5.17).

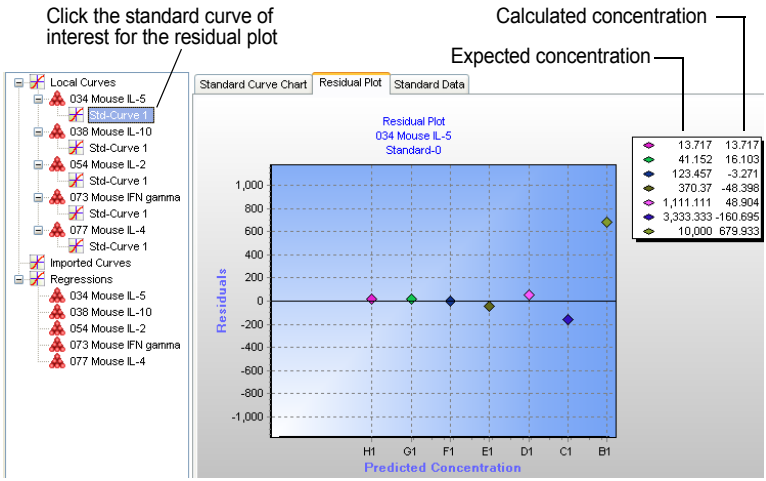


Figure 5.17 Residual plot
Each point represents the difference between the expected and calculated concentrations of the standard data for the selected analyte.

The residual plot helps you identify nonconstant variability (*heteroscedasticity*) in the data. For more information on detecting heteroscedasticity using a residual plot, see Appendix C.

Viewing Standard Data & Specifying Outliers

In the Standard Data tab, you can view information about each standard and specify outliers in a standard data set. The software ignores outliers in the calculation of the standard curve.

1. In the Standard Curves window, click the Standard Data tab.
2. Click the standard curve of interest.
   ⇒ The data for the selected standards are displayed (Figure 5.18).

Table 5.1 explains the information available in the Standard Data tab.

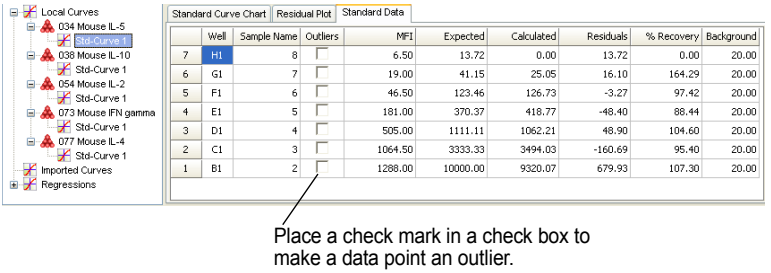


Figure 5.18 Standard Data tab

Table 5.1 Standard Data

Item	Description
Outliers	To make a standard data point an outlier, place a check mark in the check box next to the standard data point. The software ignores outliers during the calculation of the standard curve.
MFI	Mean fluorescence intensity of the data point in the selected analyte standard.
Expected	The standard concentration for the selected analyte that was entered using the autofill function (or manually).
Calculated	The standard concentration for the selected analyte that was interpolated or extrapolated from the standard curve.
Residuals	Calculated - Expected
% Recovery	(Calculated/Expected) * 100
Background	MFI background value for the plate.

3. To specify an outlier, place a check mark next to the standard data point of interest (Figure 5.18).

   Alternatively, you can select outliers in the Standard Curve Chart tab:

   a. Click the Standard Curve Chart tab.

   b. Click the **Chart Selection Tool** button, then click a point in the standard curve that you want to make an outlier.

      ⇒ The Standard Data tab highlights the selected point.

   c. Place a check mark in the highlighted outlier check box.

4. Recalculate the standard curve and analyte concentrations. (Click the **Calculate** button or select **Calculations** > **Calculate STD Curves** from the menu bar.)

5.6 Importing Standard Curves

You can import standard curves from another plate so that you can easily compare standards from different plates.

1. Open the plate that contains the standard curves for export (source plate).
2. Open the plate that the standard curves will be imported to (destination plate).
3. Click the **Import/Export std curves** button.

   ⇒ The plate windows are in import/export mode.

NOTE: The plate windows must be in import/export mode to export standard curves from the destination plate to the source plate.

4. Use the click-and-drag method to move the standard data set of interest from the source plate to the well grid of the destination plate.

   ⇒ The Standard Curves window displays a list of the standard curves imported from the source plate (Figure 5.19).

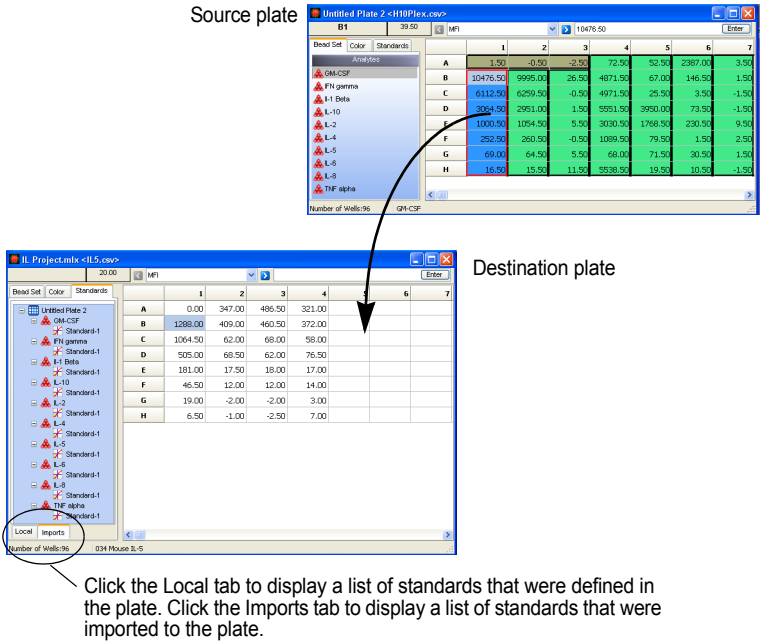

Source plate
Destination plate
Click the Local tab to display a list of standards that were defined in the plate. Click the Imports tab to display a list of standards that were imported to the plate.

Figure 5.19 Standard curves can be exported
Local and imported standard curves are listed in the Standards tab of the destination Plate window.

NOTE: To view and conveniently compare standard curves, open the Standard Curves window (click the button). For more information, see *Opening the Standard Curves Window* on page 5.16.

CHAPTER 6

VIRTUAL PLATES

A virtual plate is a software simulation of an empty microtiter plate. User-selected unknown and standard data from one or more actual plates (.csv or .mlx) can be added to a virtual plate. In a virtual plate you can:

- Compare and analyze samples from user-selected actual plates (.csv or .mlx)
- Specify custom row and column dimensions to increase analysis throughput
- Generate a Dose-Reponse curve for user-selected data

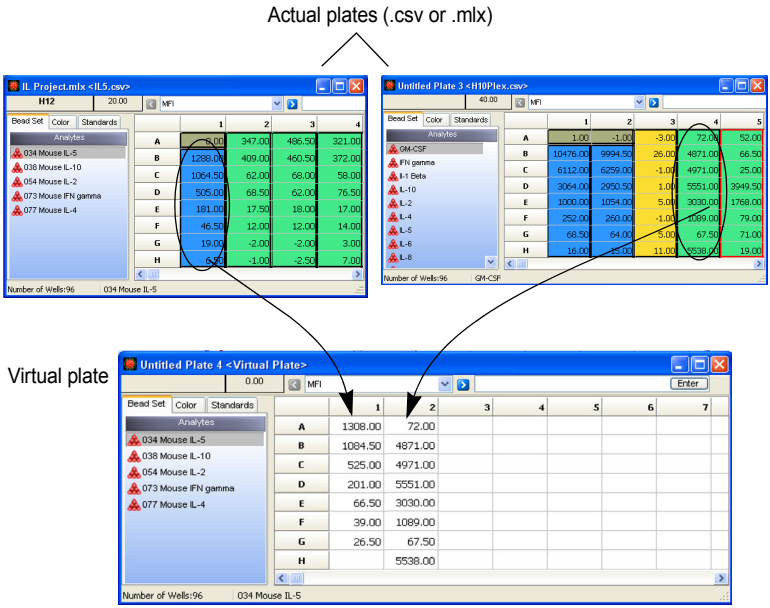


Figure 6.1 Add user-selected data from actual plates (.csv or .mlx) to a virtual plate

In a virtual plate, you can perform a regression analysis on user-selected data to generate a Dose-Reponse curve and determine the LogEC50 value.

6.1
Creating a Virtual Plate

1. Open the Luminex results files (.csv) or MasterPlex™ QT files (.mlx) that are the data sources for the virtual plate.
2. If the Plate Wizard is not open, click the **Plate Wizard** button .
   ⇒ The Plate Wizard appears (Figure 6.2).

NOTE: If the **Display wizard at start up** option is chosen, the Plate Wizard is displayed when MasterPlex™ QT starts.

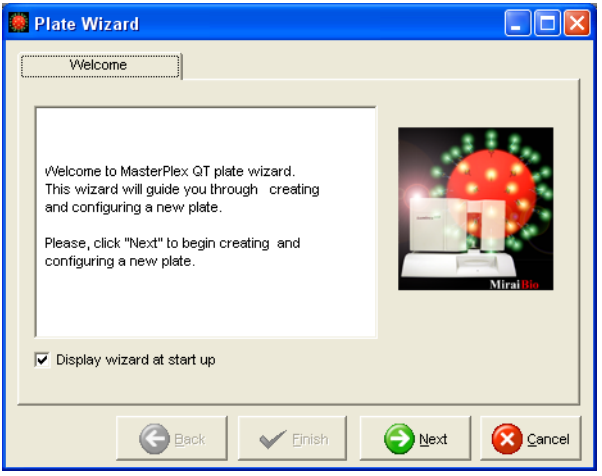


Figure 6.2 Plate Wizard, Welcome tab

3. Click **Next**.
   ⇒ The Select Plate Type tab appears (Figure 6.3).

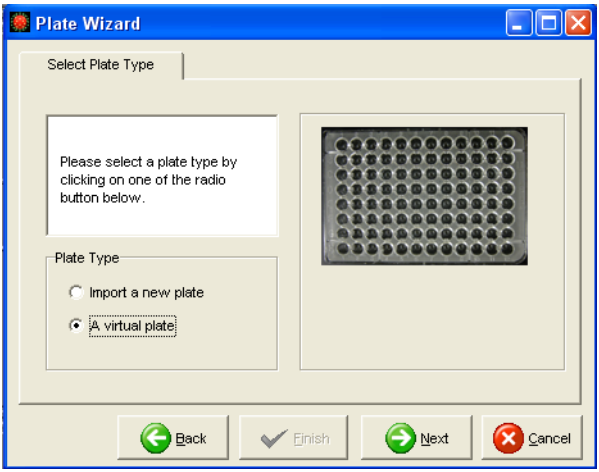
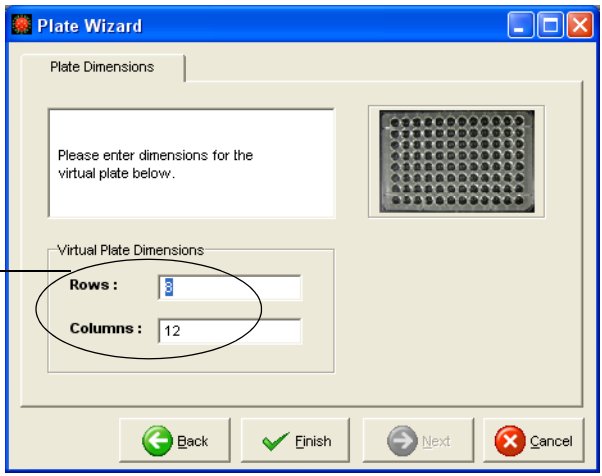


Figure 6.3 Plate Wizard, Select Plate Type tab

4. Choose the **Virtual Plate** option and click **Next**.
   ⇒ The Plate Dimensions tab appears (Figure 6.4).



The row and column defaults can be edited in the Preferences dialog box (click the button).

Figure 6.4 Plate Wizard, Plate Dimensions tab

5. Enter the number of rows and columns for the virtual plate. Click **Finish**.
   ⇒ A Plate window opens and displays the empty well grid of the virtual plate (Figure 6.5).

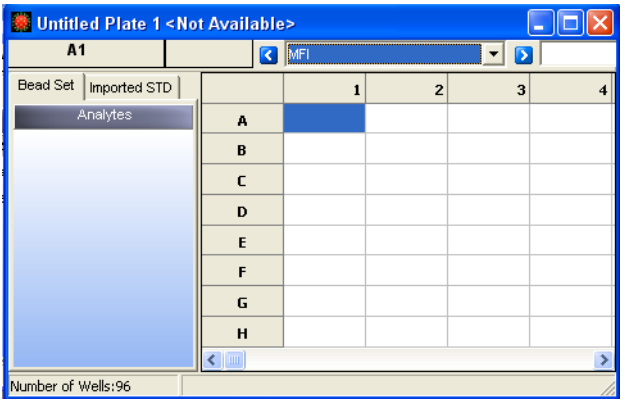


Figure 6.5 Virtual plate

Selecting Data from a Source Plate

The virtual pipette copies (*aspirates*) data from user-selected wells in a source plate and pastes (*dispenses*) the data into a virtual plate. The virtual pipette aspirates all of the analyte data in a well, including the computed analyte concentrations. It remains loaded until you dispense or clear the pipette.

NOTE: The data source plates must contain the same type and number of analytes, otherwise concentrations cannot be calculated. If the source plates contain the same number of analytes, but they are named differently, use the virtual analyte filter to rename analytes so that the nomenclature is consistent. (See *Selecting and Renaming Analytes* on page 6.9.)

1. Click the **Virtual Pipette** button .

   ⇒ The virtual pipette appears when the mouse cursor is positioned over a plate window.

2. In the source plate, select the wells of interest.

   To select adjacent wells, press and hold the mouse button while you drag the mouse pointer to select the wells of interest.

NOTE: Selecting non-adjacent wells is not recommended.

3. Right-click the selected wells and select **Aspirate** from the pop-up menu that appears (Figure 6.6).
   ⇒ The data for the analytes in the selected wells are added to the virtual pipette and is ready to dispense into a virtual plate.

NOTE: If the background is subtracted in the source plate, the virtual pipette aspirates and transfers background-subtracted values. If you do not want to aspirate background-subtracted values, make sure the background subtraction is turned off before you aspirate data into the virtual pipette. (Click the button to turn background subtraction on or off.)

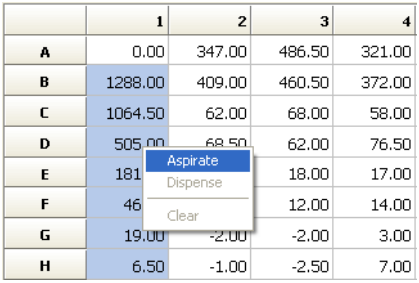


Figure 6.6 Well grid
Right-click selected wells to display the pop-up menu.

4. To clear the data from the virtual pipette, right-click and select **Clear** from the pop-up menu.

Adding Data to a Virtual Plate

After the virtual pipette aspirates data from the source plate, it is ready to dispense the data into the virtual plate.

1. Position the virtual pipette over the virtual plate.
2. Click the first well to which the data will be added.
3. Right-click the well and select **Dispense** from the pop-up menu that appears.

   ⇒ The data are added to the virtual plate (in the same configuration as in the source plate) (Figure 6.7).

NOTE: If the number or names of the analytes in the virtual pipette is different from that in the virtual plate, the virtual analyte filter automatically appears. For more information on using the filter, see *Selecting and Renaming Analytes* on page 6.9.

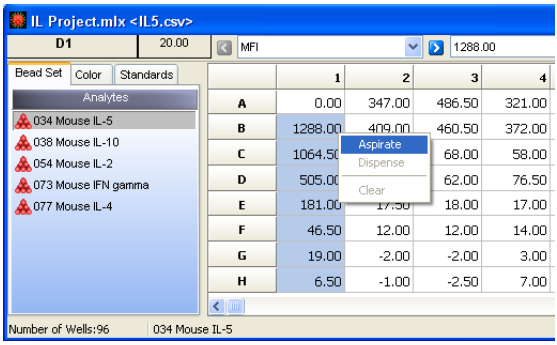
NOTE: Data in a virtual plate cannot be removed, but can be overwritten.

4. After you finish adding data to the virtual plate, click the **Virtual Pipette** button to turn off the virtual pipette.

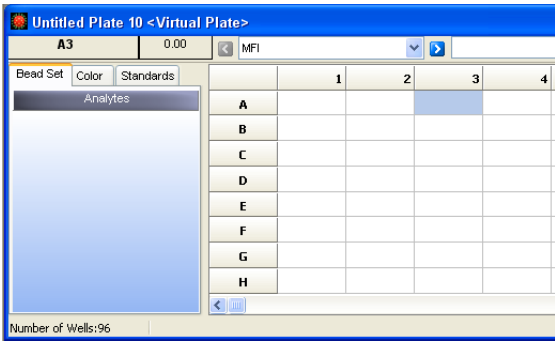
1. Open a .mlx or .csv.

   Click the button to turn on the virtual pipette.

2. Select the wells of interest in the source plate (.csv or .mlx). Right-click the selected wells and choose aspirate from the pop-up menu.



2. In the virtual plate, select the first well where you want to dispense the data.



3. Right-click the well and select **Dispense** from the pop-up menu. The data are added to the virtual plate (starting at the selected well) in the same configuration as in the source plate.

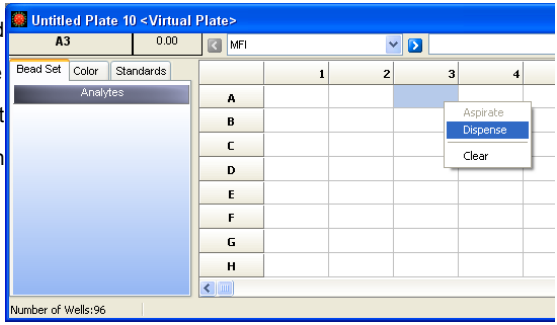


Figure 6.7 Adding data to a virtual plate

Open a source plate (.mlx or .csv) and create a virtual plate (click the button to start the virtual plate wizard).

6.2
Working With the Virtual Analyte Filter

In a multiplex assay, all of the plate wells must contain:

- the same types of analytes (bead sets) with the same nomenclature
- the same number of analytes

This is true for virtual plates as well. When you add data to a virtual plate, MasterPlex™ QT compares the name and number of the analytes in the virtual pipette to those in the virtual plate. The virtual pipette will not dispense if there are discrepancies between the number or names of analytes in the pipette and the virtual plate. If the number of analytes in the pipette is greater than that in the destination plate, the virtual analyte filter automatically appears (Figure 6.9).

The virtual analyte filter displays a list of the analytes that are present in the virtual pipette. It enables you to choose the analytes that you want to add to the virtual plate and, if necessary, rename them to be consistent with the number and name of analytes in the virtual plate.

If you add data to a virtual plate from source wells that contain different analyte names or a different number of analytes, data holes are created. As a result, a well in the virtual plate appears blank if the analyte selected in the analyte panel is not present in the well. If a plate (.csv, .mlx, or virtual) contains data holes, the concentrations cannot be calculated.

NOTE: In order to prevent data holes, if the number of analytes in the virtual pipette is less than the number of analytes in the destination plate, the data cannot be added to the virtual plate.

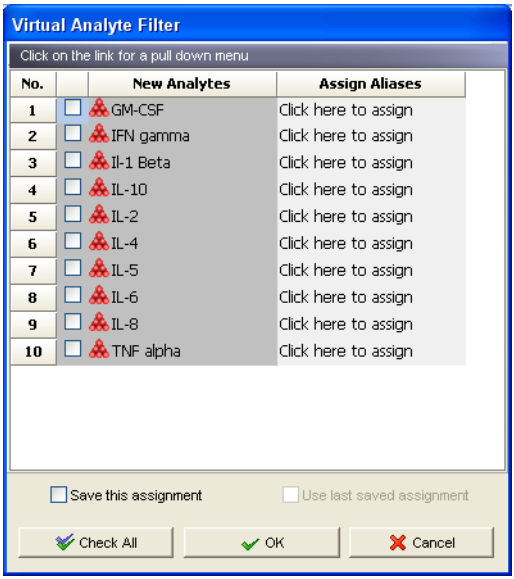

Virtual Analyte Filter
Click on the link for a pull down menu
No.
New Analytes
Assign Aliases
1 GM-CSF Click here to assign
2 IFN gamma Click here to assign
3 Il-1 Beta Click here to assign
4 IL-10 Click here to assign
5 IL-2 Click here to assign
6 IL-4 Click here to assign
7 IL-5 Click here to assign
8 IL-6 Click here to assign
9 IL-8 Click here to assign
10 TNF alpha Click here to assign
Save this assignment
Use last saved assignment
Check All
OK
Cancel

Figure 6.8 Virtual analyte filter shows the analytes in the virtual pipette

Selecting and Renaming Analytes

If the virtual analyte filter appears, you must select and, if necessary, rename the analytes to match the number and names of the analytes in the virtual plate.

1. In the virtual analyte filter (Figure 6.9), place a check mark next to each analyte that you want to add to the virtual plate. To select all analytes for the virtual plate, click **Check All**.
2. To rename an analyte so that it is consistent with the nomenclature in the virtual plate:
   a. Click **here to assign** next to the analyte that you want to rename.
      ⇒ A drop-down list shows the names of the analytes in the virtual plate (Figure 6.9).
   b. Select a name from the drop-down list.
      ⇒ The virtual analyte filter displays the new name for the analyte.

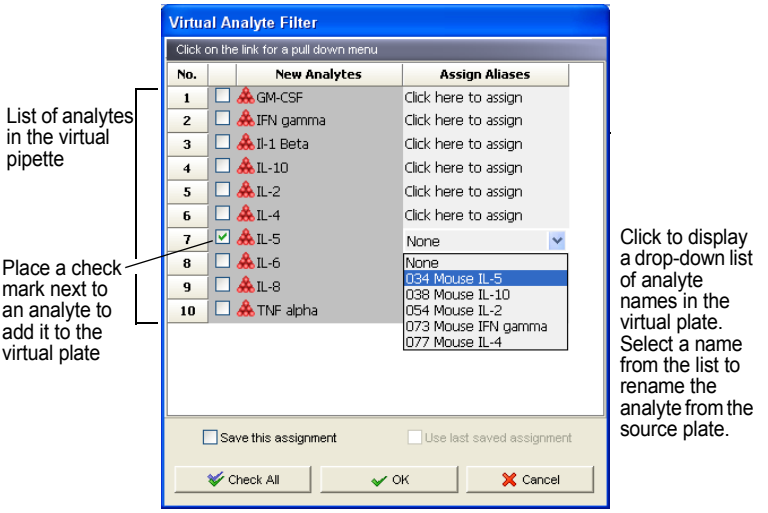

List of analytes in the virtual pipette
Place a check mark next to an analyte to add it to the virtual plate
Virtual Analyte Filter
Click on the link for a pull down menu
No.
New Analytes
Assign Aliases
1 GM-CSF Click here to assign
2 IFN gamma Click here to assign
3 Il-1 Beta Click here to assign
4 IL-10 Click here to assign
5 IL-2 Click here to assign
6 IL-4 Click here to assign
7 IL-5 None
8 IL-6
9 IL-8
10 TNF alpha
None
034 Mouse IL-5
038 Mouse IL-10
054 Mouse IL-2
073 Mouse IFN gamma
077 Mouse IL-4
Save this assignment
Use last saved assignment
Check All
OK
Cancel
Click to display a drop-down list of analyte names in the virtual plate. Select a name from the list to rename the analyte from the source plate.

Figure 6.9 Virtual analyte filter

3. To save the renaming assignments for use again with the same source plate (.csv or .mlx) during the current session, choose the **Save this assignment** option.

   If you want to aspirate other data from the same source plate, choose the **Use last saved assignments** option in the virtual analyte filter to automatically rename all of the analytes in the filter.

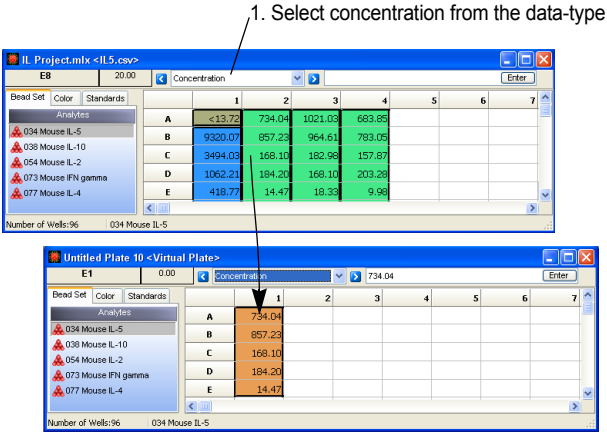
4. Click **OK**.

   ⇒ The data are added to the virtual plate and the virtual analyte filter closes.

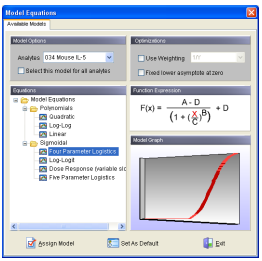
6.3
Generating a Dose-Reponse Curve

A Dose-Reponse curve plots the dose of an agent (independent variable) on the x-axis and the response (computed concentration) on the y-axis. After you have created a virtual plate that contains the concentration data of interest, you can run a regression analysis on the data in the virtual plate to generate a Dose-Reponse curve (Figure 6.10). (For more information on Dose-Reponse curves, see *Appendix D, Dose-Response Analysis* on page D.1.)

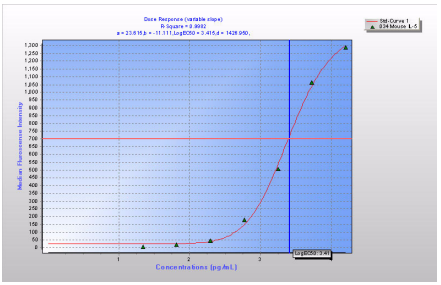
The software determines the LogEC50 value from the Dose-Reponse curve. The logEC50 value is the log of the agent concentration (for example, a drug or other chemical) that produces a response half way between the baseline and maximum response.



2. Create a virtual plate.
3. Transfer response data to the virtual plate using the virtual pipette.
4. Mark and group wells into a regression data set.
5. Select Standard/Independent values from the data-type drop down list and enter the agent concentrations (use the Autofill function or enter manually).



6. To select a Dose-Reponse model, right-click the regression data set and select **Assign Model Equation**.
7. Click the **Regression Analysis** button .



8. To view the Dose-Reponse curve, click the **Standard Curves** button .

Figure 6.10 Steps to generate a Dose-Reponse curve

1. Create a virtual plate and use the virtual pipette to add the data for the Dose-Reponse curve to the virtual plate. (See "Creating a Virtual Plate" on page 6.2.)

NOTE: After you finish creating the virtual plate, click the button to turn off the virtual pipette.

2. Select the wells that you want to include in the regression analysis data set.

   The regression analysis data set includes the wells that contain the response data to different dosages of the agent.

3. Right-click the selection and choose **Mark Wells > Regression** from the pop-up menu that appears. Alternatively, click the **Mark regression wells** button or select **Template > Mark Wells > Mark Regression Wells** from the menu bar.
4. Right-click the regression data set and select **Group Wells** from the pop-up menu. Alternatively, click the **Group** button or select **Template > Group Wells** from the menu bar.
5. If the agent was serially diluted, follow these steps to enter the agent concentration using the auto fill function:
   a. Choose **Standard/Independent Values** from the data type drop-down list.
   b. Right-click the regression data set and select **Auto Fill** from the pop-up menu that appears. Alternatively, click the **Auto Fill** button .

   ⇒ The Auto Fill dialog box appears (Figure 6.11).

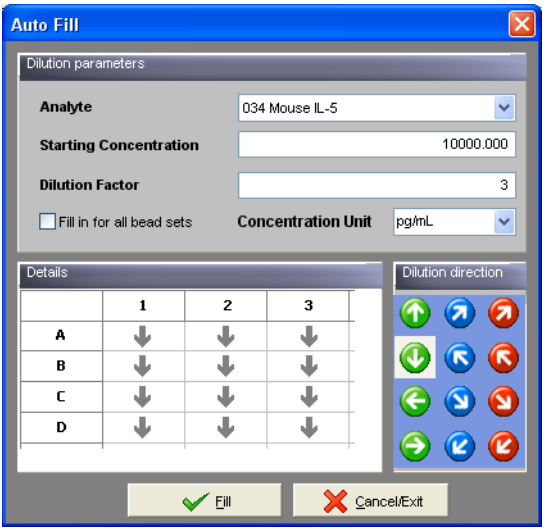


Figure 6.11 Auto Fill dialog box
Use the auto fill function to set concentrations for a serially diluted agent.

c. Enter the starting concentration for the analyte displayed in the Analyte drop-down list.
d. Enter the dilution factor
e. If the starting concentration and dilution factor are the same for all analytes, choose the **Fill in for all bead sets** option. If not, make another selection from the Analyte drop-down list and repeat step b and step c.
f. Click **Fill** after the starting concentration and dilution factor are entered for all analytes.

6. If the agent was not serially diluted, follow these steps to manually enter the agent concentration for each analyte:

a. Click the **Edit mode** button .
b. Choose **Standard/Independent** from the data type drop-down list.
c. Select an analyte in the Analyte panel.
d. Click a well in the regression data set and enter the agent concentration in the edit box.
e. Click **Enter**.
⇒ The value is displayed in the well grid.
f. Repeat step b to step e for the remaining analytes.

7. Right-click the regression data set and select **Assign Model Equation** from the pop-up menu that appears. Alternatively, click the regression data set and click the **Select Model** button .
   ⇒ The Model Equations dialog box appears (Figure 6.12).

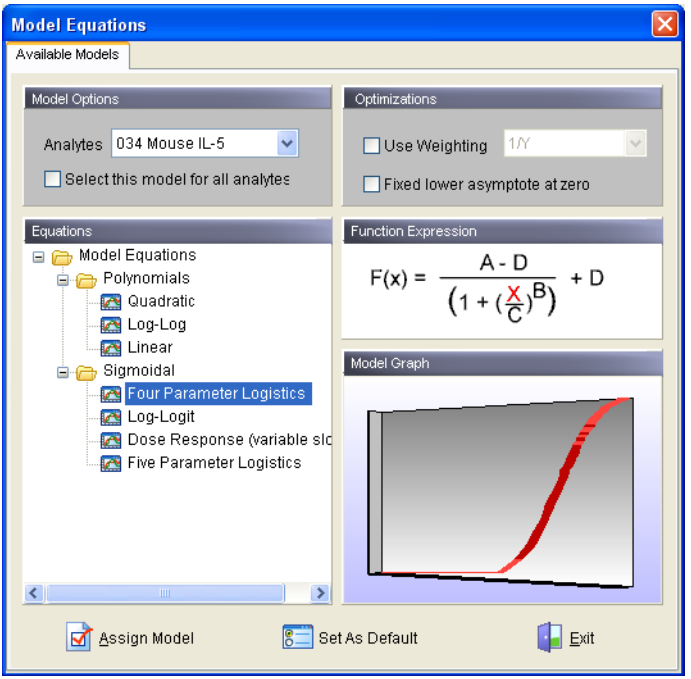


Figure 6.12 Model Equations dialog box
Select a model for the Dose-Reponse curve

8. Select the Dose-Reponse (variable slope) model equation and choose the **Select this model for all analytes** option.
9. Click **Assign Model**.
10. Click the **Regression Analysis** button .
    ⇒ The software computes the Dose-Reponse curve and displays a confirmation message when the calculation is completed.
11. To view the Dose-Reponse curve, click the **Standard Curves** button .

    The Dose-Reponse curve shows the agent dose (independent variable) on the x-axis and the response (interpolated concentration) on the y-axis (Figure 6.13). It displays the parameters of the model equation and the LogEC50 value.

12. To compute the EC50 value, take the antilog of the LogEC50.

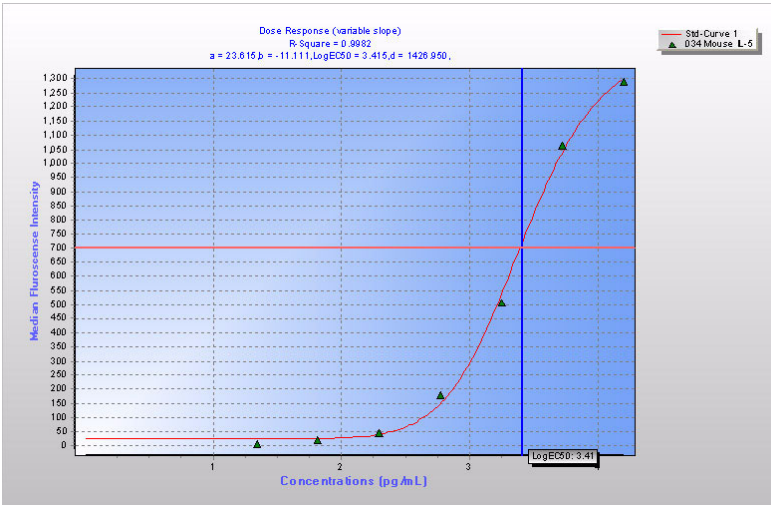


Figure 6.13 Dose-Reponse curve

Dose (independent variable) on the x-axis and response (computed concentration, dependent variable) on the y-axis.

MasterPlex™ GT displays MFI, count, or concentration data in several graph formats in the Data Chart window.

7.1 Viewing a Data Chart

1. In the Plate window, select one or more wells of interest.

   To select adjacent wells, press and hold the mouse button while you drag the mouse pointer to select the wells of interest. To select non-adjacent wells, press and hold the **Ctrl** key while you click the wells of interest.

2. Click the **Open Data Charts** button . Alternatively, select **Plate > Data Charts** from the menu bar.

   ⇒ The Data Chart window opens and displays the well chart(s) (default) for the selected well(s) (Figure 7.1).

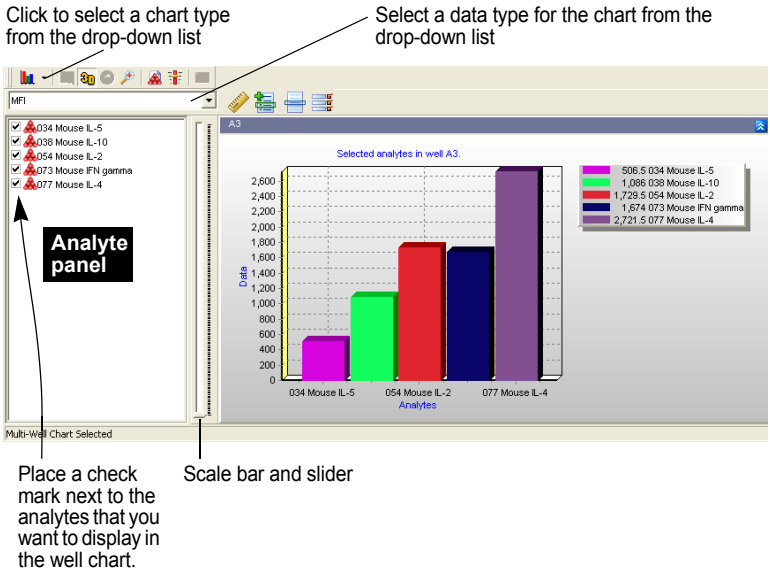


Figure 7.1 Multi-well chart
MFI data for the selected analytes in well B1

3. To display another graph format for the selected wells, click the button and make a selection from the drop-down list.

⇒ The well data are displayed in the selected format.

4. To change the data type in the chart (MFI, count, or concentration), make a selection from the data drop-down list (Figure 7.1).
5. To remove an analyte from a chart, remove the check mark next to the analyte name in the analyte panel.
6. To view other well data in the current chart format:
   a. Return focus to the Plate window.
   b. Select the well(s) of interest.
   c. Click the Data Chart window.
      ⇒ The Data Chart window is updated and displays the new well data in the selected format.
7. To change the y-axis scale, move the slider up or down the scale bar.

7.2 Data Chart Types

MasterPlex QT provides seven different chart types (Table 7.1). To choose a chart format for the selected well data, click the button, and make a selection from the drop-down list of data charts.

NOTE: The data chart toolbar is available if a Data Chart window is open.

Table 7.1 MasterPlex™ QT data charts

Data Chart	Displays...	See Page
Multi-Well Chart	Separate bar graphs of analyte data for each user selected well.	7.4
3D Depth Chart	Bar graph of analyte data that includes all selected wells. Analytes from a particular well are organized by row.	7.6
Inverted 3D Depth Chart	Bar graph of analyte data that includes all selected wells. Analytes from a particular well are organized by column.	7.7

Table 7.1 MasterPlex™ QT data charts

Data Chart	Displays...	See Page
Intensity Map	A qualitative map of the intensity data for the analytes in one well. The map colors indicate the relative intensity of an analyte compared to the other analytes in the same well.	7.8
Single Analyte Chart	Bar graph of a single analyte across all user selected wells.	7.10
Stacked Analyte Chart	Bar graph of user selected wells. Each color-coded segment of a bar represents the contribution of an analyte to the total data value for the well.	7.11
Stacked Well Chart	Bar graph of analytes. Each color-coded segment of a bar represents the contribution from a well to the total data value for the analyte.	7.12

Multi-Well Chart

The multi-well chart displays a bar graph of analyte data (MFI, count, or concentration) for a user-selected well(s). Each bar represents an analyte. The multi-well chart is the default format that is displayed when you open the Data Chart window.

1. To minimize a multi-well, click the arrow (Figure 7.2).
2. To restore a chart, click the arrow.

   Alternatively, click the button to minimize all charts (Figure 7.3). Click the button again to restore all charts.
3. To normalize the data for all open multi-well charts, click the **Normalize** button .

   ⇒ The well data are plotted using the y-axis scale from the chart with the largest y-axis value.

NOTE: You can choose different colors for the chart bars in the Color tab of the Plate window (see ***Plate Window Components*** on page 3.9).

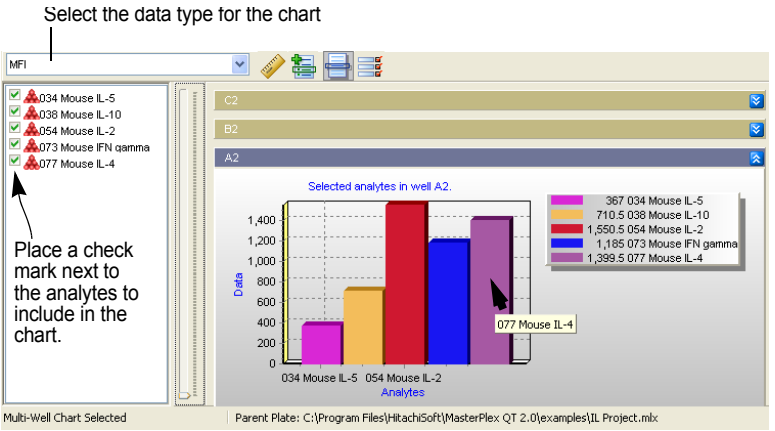


Figure 7.2 Multi-well chart for A2
Multi-well charts for C2 and well B2 are minimized. Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

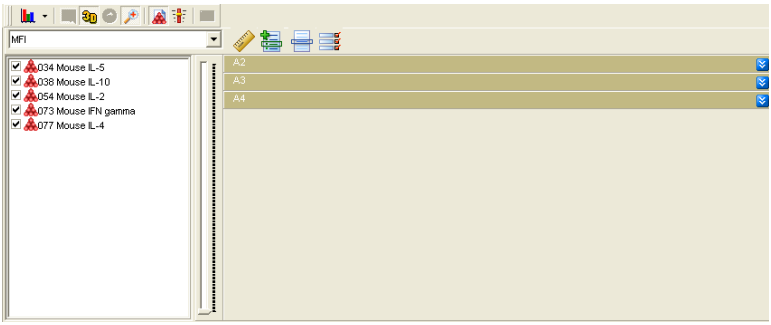


Figure 7.3 Multi-well charts are minimized

TIP: You can conveniently reorder the multi-well charts in the Data Chart window by dragging a chart to the desired position in the list.

3D Depth Chart

The 3D depth chart is a convenient way to compare the MFI, count, or concentration data in one well and across different wells (Figure 7.4). Each bar represents an analyte. The analytes of a particular well are identified by color and row.

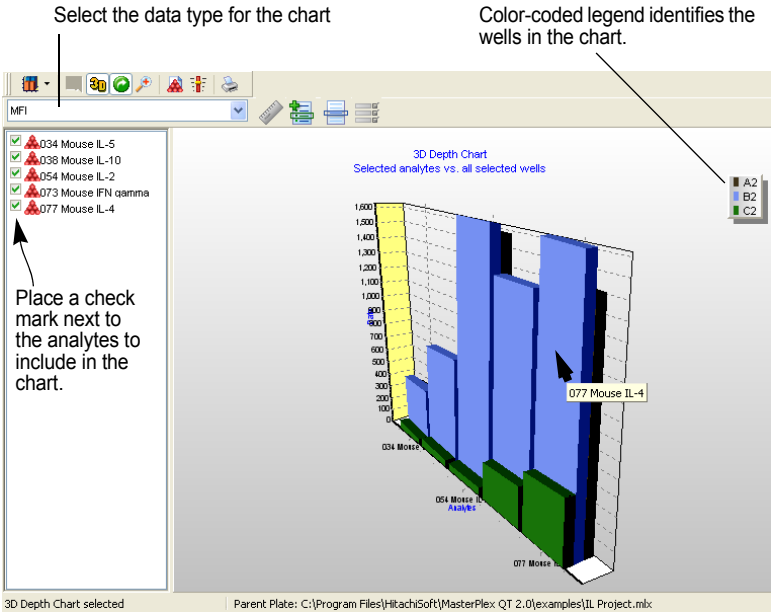

Select the data type for the chart
Color-coded legend identifies the wells in the chart.
MFI
034 Mouse IL-5
038 Mouse IL-10
054 Mouse IL-2
073 Mouse IFN gamma
077 Mouse IL-4
Place a check mark next to the analytes to include in the chart.
3D Depth Chart
Selected analytes vs. all selected wells
A2
B2
C2
077 Mouse IL-4
3D Depth Chart selected
Parent Plate: C:\Program Files\HitachiSoft\MasterPlex QT 2.0\examples\IL Project.mlx

Figure 7.4 3D depth chart compare analyte data in the same well or between different wells.

Each bar represents an analyte. Each row of color-coded bars represents the analyte data from one well. Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

Inverted 3D Depth Chart

Each bar of the chart represents an analyte in a selected well. The bars are organized into columns and each column represents the analytes in respective selected wells (Figure 7.5).

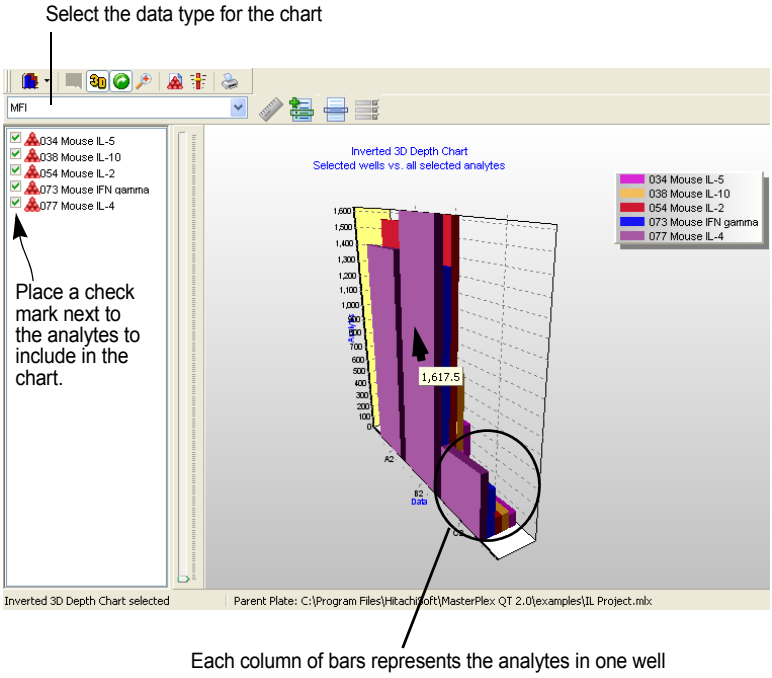

Select the data type for the chart
MFI
034 Mouse IL-5
038 Mouse IL-10
054 Mouse IL-2
073 Mouse IFN gamma
077 Mouse IL-4
Place a check mark next to the analytes to include in the chart.
Inverted 3D Depth Chart
Selected wells vs. all selected analytes
1,617.5
Inverted 3D Depth Chart selected
Parent Plate: C:\Program Files\Hitachi Soft\MasterPlex QT 2.0\examples\IL Project.mlx
Each column of bars represents the analytes in one well

Figure 7.5 Inverted 3D depth chart

Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

Intensity Map

The intensity map is a qualitative picture of the intensity data in the wells selected in the Plate window. The map provides a convenient way to look at a large number of samples and identify analytes that consistently have a high (or low) intensity compared to the other analytes in the same well.

NOTE: The intensity map compares and ranks data in the same well. It does not compare data across wells.

Figure 7.6 Intensity map

Click the map to display a tool tip (analyte name or y-axis data). Right-click the map for tool tip options.

The intensity map is color-coded. Figure 7.6 shows the default colors:

- Red = peak intensity
- Green = mid-range intensity
- Blue = low intensity

The default colors may be changed. (See *Intensity Map Settings* on page 7.35.)

Each row in the map represents the relative intensities of the analytes in one well.

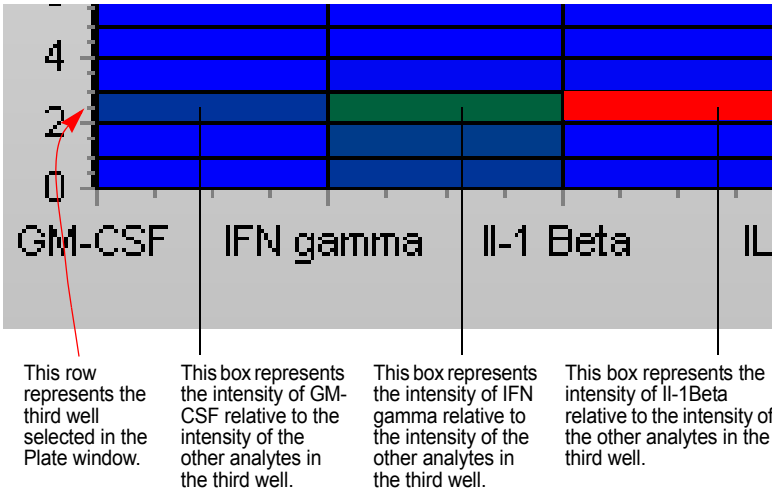

4
2
0
GM-CSF
IFN gamma
Il-1 Beta
IL
This row represents the third well selected in the Plate window.
This box represents the intensity of GM-CSF relative to the intensity of the other analytes in the third well.
This box represents the intensity of IFN gamma relative to the intensity of the other analytes in the third well.
This box represents the intensity of Il-1Beta relative to the intensity of the other analytes in the third well.

Figure 7.7 Intensity map
In this example map, Il-1Beta has the highest intensity and GM-CSF has the lowest intensity in the third well that was selected in the Plate window.

Single Analyte Chart

This chart shows the data (MFI, count, or concentration) for a single analyte across all user-selected wells (Figure 7.8). Each bar represents the selected analyte data from one well.

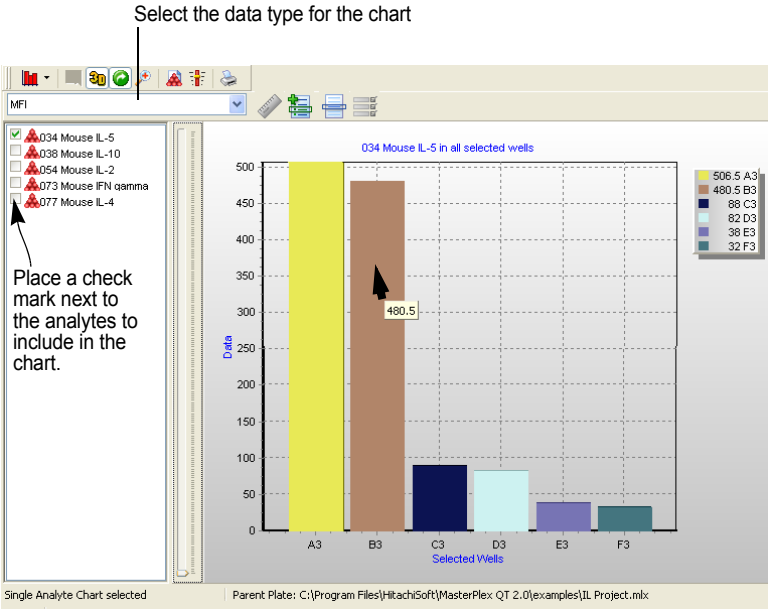


Figure 7.8 Single analyte chart

Each bar represents data for a single analyte from one well. Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

Stacked Analyte Chart

Each bar represents a single well. The color-coded segments of a bar represent the contribution of each analyte to the total MFI, count, or concentration value in the well (Figure 7.9).

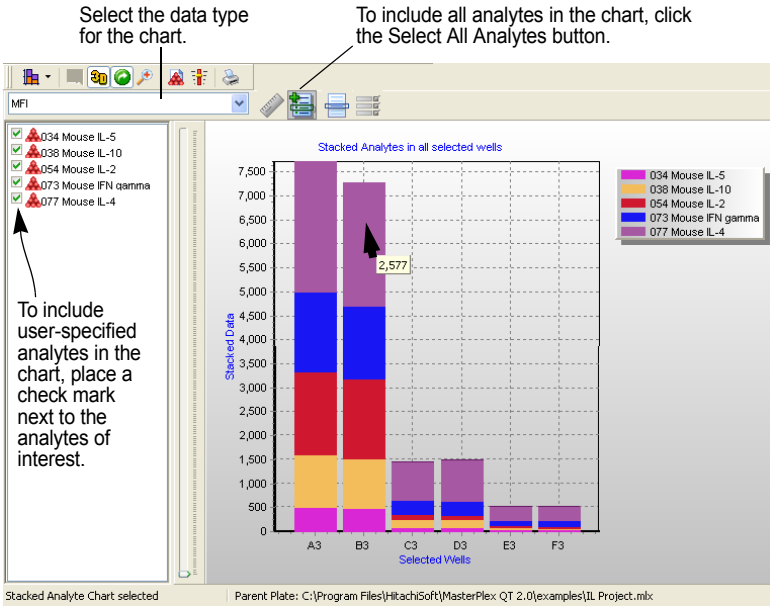


Figure 7.9 Stacked analytes chart

Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

Stacked Well Chart

Each bar in the stacked well chart represents the sum total of the MFI, count, or concentration values of an analyte across the user-selected wells. The color-coded segments of a bar show the contribution of each well to the total value.

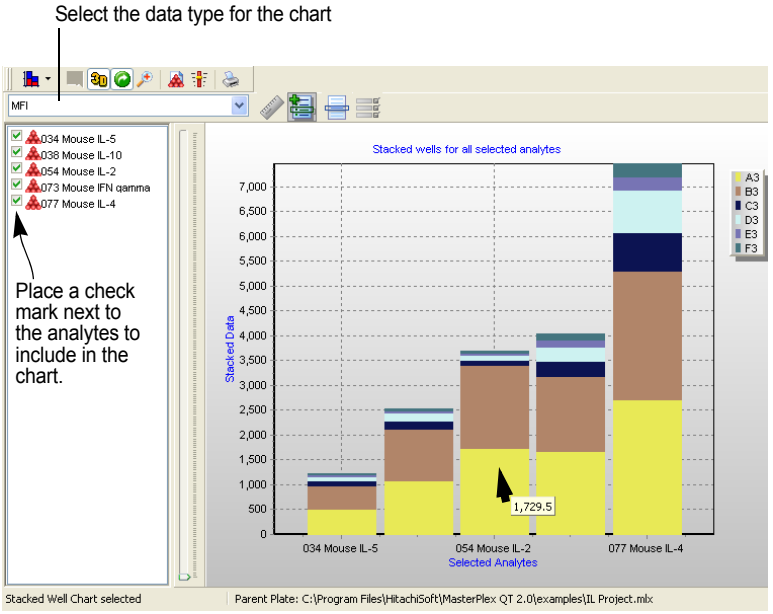


Figure 7.10 Stacked well chart
Click a bar to display a tool tip (analyte name or y-axis data). Right-click a bar for tool tip options.

7.3
Working With Data Charts

Table 7.2 explains the toolbar button functions in the Data Chart window. The available buttons depend on the type of data chart that is open.

Table 7.2 Toolbar buttons in the Data Chart window

Button	Click to...
	Choose another data chart format. The software plots the data for the selected wells in the new format.
	Toggle the chart display between a two and three dimensional view.
	Put the chart in rotate mode so that you can rotate the chart around the x or y-axis.
	Put a chart in zoom mode so that you can magnify a user-selected area of the chart.
	Hide or show the analyte panel.
	Hide or show the scale bar. To change the y-axis scale, move the scale slider up or down the scale bar.
	Opens the Chart Print Preview window so a chart may be printed.
	Normalize the data for the open multi-well charts. Plots the selected well data using the y-axis scale from the chart with the largest y-axis value. Note: This function is only available for the multi-well chart.
	Select all analytes in the analyte panel for display in the chart.
	Removes all analytes from the chart.
	Minimize or restore all well charts in the Data Chart window.

Copying, Saving, or Sending a Chart

You can copy a chart to the system clipboard or save a chart.

1. Open the chart of interest.
2. Click the **Export Chart** button . Alternatively, select **Edit** > **Export Chart** from the menu bar.
   ⇒ The Export Chart dialog box appears (Figure 7.11).

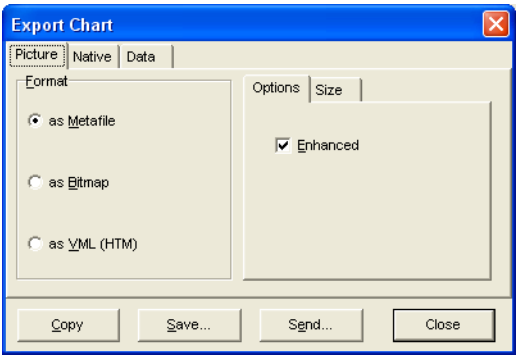


Figure 7.11 Export Chart dialog box

3. In the Picture tab, choose a file format option:

   Metafile saves the chart in a scalable .wmf format. Choose the **Enhanced** option to save the charts in enhanced metafile format (.emf).

   Bitmap saves the chart in .bmp format. Depending on the chart dimensions, the .bmp file size can become large.

   VML (HTM) saves the chart as an HTML document (.htm) that can be viewed using a web browser.

4. To specify a size option for the chart:
   a. Click the Size tab.
   b. Enter the width and height (in pixels) for the chart.
   c. Choose the **Keep aspect ratio** option to maintain the same dimension ratio at the new chart size.
5. To copy the chart, click **Copy**.
6. To save the chart, click **Save**.
   ⇒ The Save As dialog box appears (Figure 7.12).

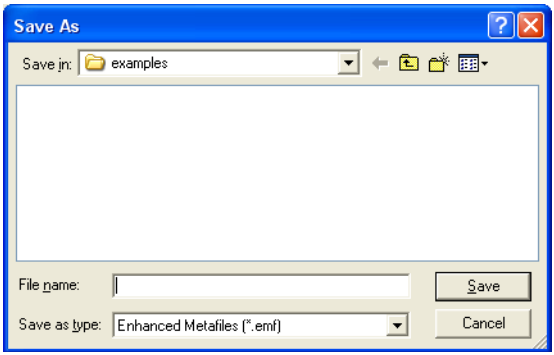


Figure 7.12 Save As dialog box

7. To e-mail the chart:
   a. Click **Send**.
   ⇒ The Choose Profile dialog box appears (Figure 7.13).

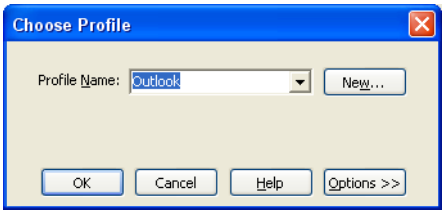


Figure 7.13 Choose Profile dialog box

   b. Select a recipient for the e-mail and click **OK**.

NOTE: This dialog is only functional with Microsoft Outlook.

Copying or Saving Data

The software can export data from a chart to other applications. The data may be copied to the system clipboard or saved in different file formats.

1. Open the chart of interest.
2. Click the **Export Chart** button . Alternatively, select **Edit** > **Export Chart** from the menu bar.
   ⇒ The Export Chart dialog box appears (Figure 7.11).
3. Click the Data tab.

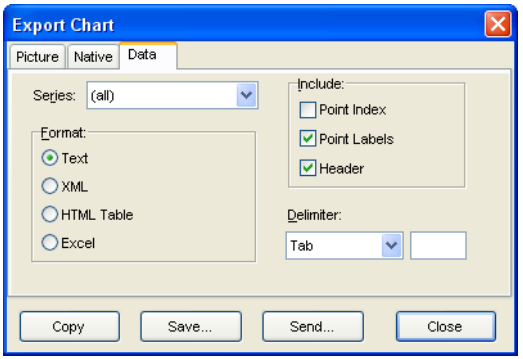


Figure 7.14 Export Chart dialog box, Data tab

4. Make a selection from the **Series** drop-down list. To choose the data from all the wells in the chart, select **All**.
5. Choose a file format:

   Text option saves the data is plain ASCII text format (Figure 7.16).

   XML option saves the data in extensible markup language.

   HTML Table option saves the data to a table in an HTML file.

   Excel option saves the data in .xls file format. Copying to the system clipboard is not available for this option.
6. If you are exporting data to a text file, make a selection from the Delimiter drop-down list. The delimiter specifies how data values are separated.

7. If desired, choose the **Point Index**, **Point Labels**, or **Header** options.

Point Index	Numbers the members of the data series.
Point Labels	Displays the analyte name for each series.
Header	Displays a header for each column of the data series.

8. To copy the data series to the system clipboard, click **Copy**.
9. To save the data to a file:
   a. Click **Save**.
      ⇒ The Save As dialog box appears (Figure 7.15).
   b. Enter a name for the file.
   c. Select a file format from the Save as type drop-down list.
   d. Click **Save**.

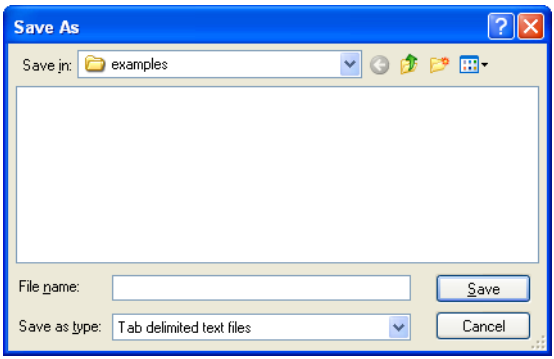


Figure 7.15 Save As dialog box

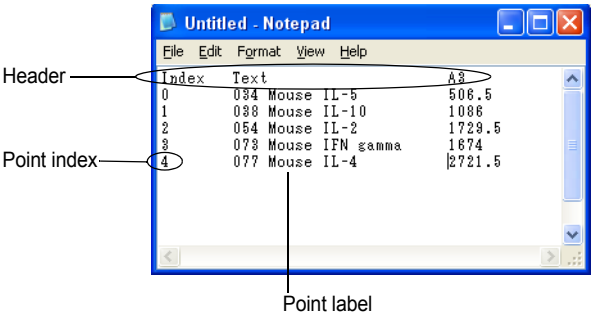


Figure 7.16 Example of data (series) for well A3 saved to a text file

Printing a Chart

1. Click the **Print current chart** button .
   ⇒ The Chart Print Preview window opens (Figure 7.17).

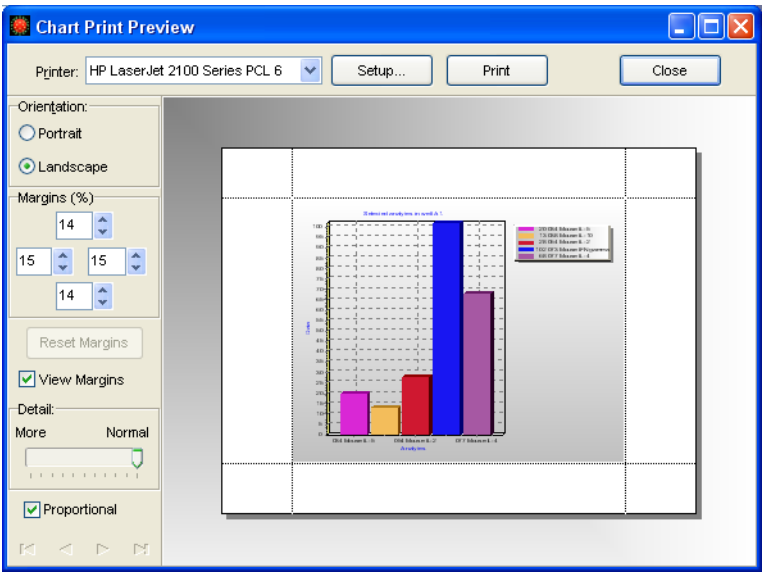


Figure 7.17 Chart Print Preview window

2. To print the chart, click **Print**.

Rotating or Magnifying a Chart

1. To rotate the chart around the x or y-axis:
   a. Click the **Chart rotate tool** button .
   b. Press and hold the mouse button while you move the mouse pointer to rotate the graph.
2. To zoom in on a chart area:
   a. Click the **Chart zoom** button .
   b. Press and hold the mouse button while you draw a rectangle (from the upper left to lower right corner) over the graph area that you want to magnify.
3. To zoom out and display a chart without magnification, press and hold the mouse button while you draw a rectangle (from the lower right to upper left corner) over the graph.

Displaying Chart Tool Tips

1. To show a tool tip that displays information in a bar chart:
   a. Click the bar of interest.
      ⇒ A tool tip displays the analyte name.
   b. To change the tool tip display, right-click a well and select **Mouse Tip Styles > Y Values** from the pop-up menu that appears (Figure 7.19).
      ⇒ The tool tip displays the MFI, concentration, bead count value for the well (depending on the data type selected in the analyte panel)
2. To change the tool tip display, right-click a well and select **Mouse Tip Styles > Y Values** from the pop-up menu that appears (Figure 7.19).
   ⇒ The tool tip displays the MFI, concentration, bead count value for the well (depending on the data type selected).

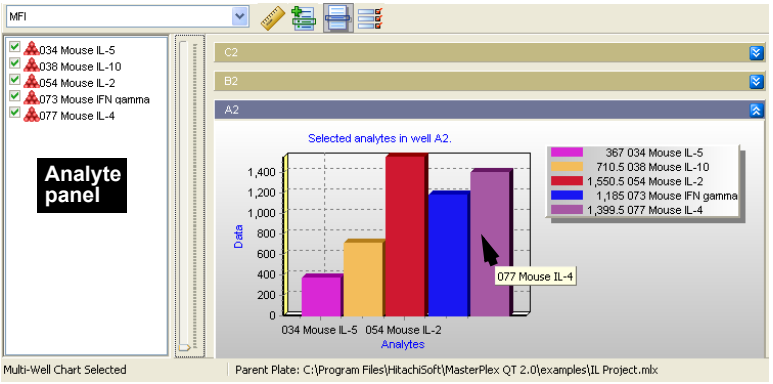


Figure 7.18 Multi-well chart
Click a bar to display a tool tip.

Select the data type for the chart y-axis (MFI, bead count, or concentration.

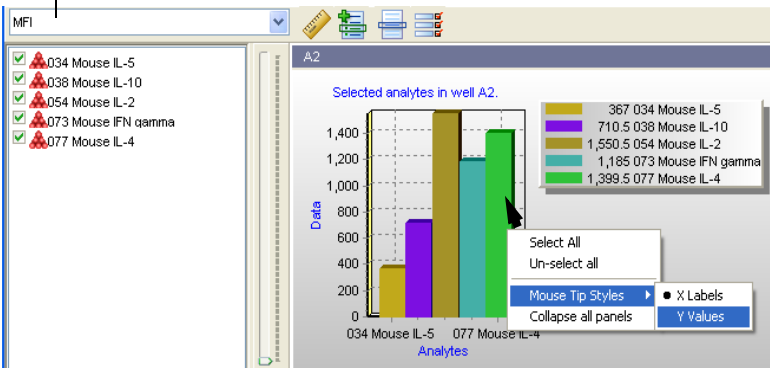


Figure 7.19 Multi-well chart
Right-click a bar to display a pop-up menu of options.

Modifying Chart Properties

You can modify the appearance of a chart.

- Click the **Chart properties** button . Alternatively, select **Chart** > **Chart Properties** from the menu bar.

  ⇒ The Chart Properties dialog box appears and displays a list of the charts in the Data Chart window (Figure 7.20).

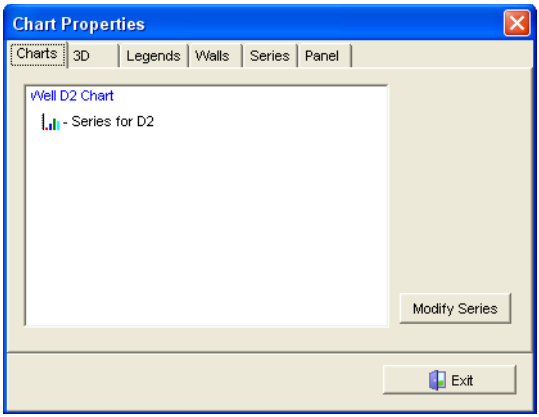


Figure 7.20 Chart Properties dialog box

NOTE: The types of chart property options available in each tab depends on the type of chart that is open.

Charts Tab

Alternative formats are available for the multi-well chart.

1. To view the available multi-well chart formats, click **Modify Series** (Figure 7.20).
   ⇒ The Chart Gallery appears (Figure 7.21).

NOTE: The **Modify Series** button is only available is a multi-well chart is open in the Data Chart window.

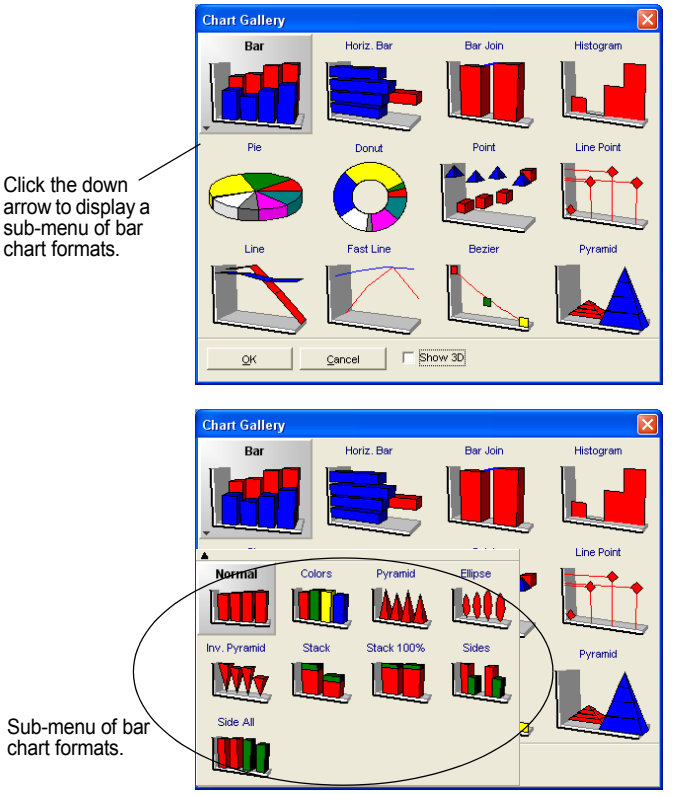


Figure 7.21 Chart gallery of available formats for the multi-well chart

2. To change the chart format, click the format of interest.
3. To view a sub-menu of styles for the selected format, click the down arrow (Figure 7.21).

4.
5. If you want to display the chart format in 3 dimensions, select the **Show 3D** option.
6. Click **OK**.

   ⇒ The selected format is applied to the multi-well chart.

3D Tab

To view 3D chart properties, click the 3D tab in the Chart Properties dialog box (Figure 7.22).

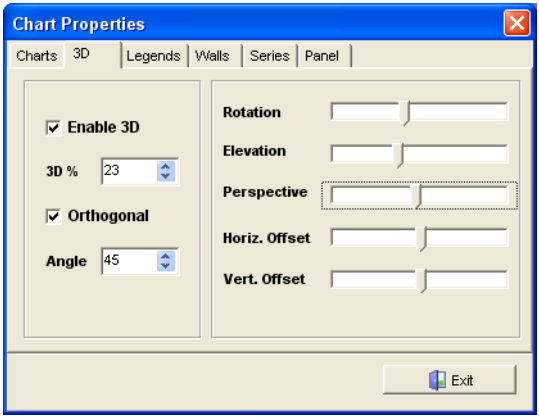


Figure 7.22 Chart Properties dialog box, 3D tab

User-Modifiable 3D Chart Properties

Enable 3D	Choose this option to display a three dimensional view of the chart.
3D%	Increases or decreases the three dimensional thickness of the graph bars.
Orthogonal	Choose this option to display a front view of the chart (no rotation around the vertical axis).
Angle	Enter a value to change the display angle for the graph bars when the orthogonal option is chosen.
Rotation	Move the slider to rotate the chart around its vertical axis.
Elevation	Move the slider to rotate the chart around its horizontal axis.
Perspective	Move the slider to change the perspective angle of the chart. The perspective effect is the dimensional appearance of the chart with respect to distance from the viewer. If this option is set to zero, the perspective option is disabled. Note: If the orthogonal option is chosen, the chart perspective cannot be changed.
Horiz. Offset	Move the slider to adjust the horizontal position of the chart in the Data Chart window.
Vert. Offset	Move the slider to adjust the vertical position of the chart in the Data Chart window.

Legends Tab

To view the chart legend properties, click the Chart tab in the Chart Properties dialog box (Figure 7.23). These settings determine the location and appearance of the legend in a data chart (Figure 7.24).

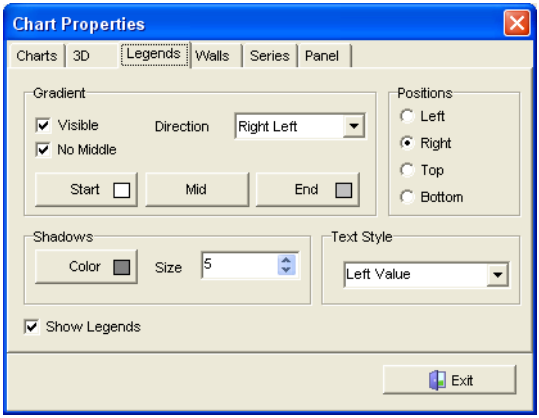


Figure 7.23 Chart Properties dialog box, Legends tab

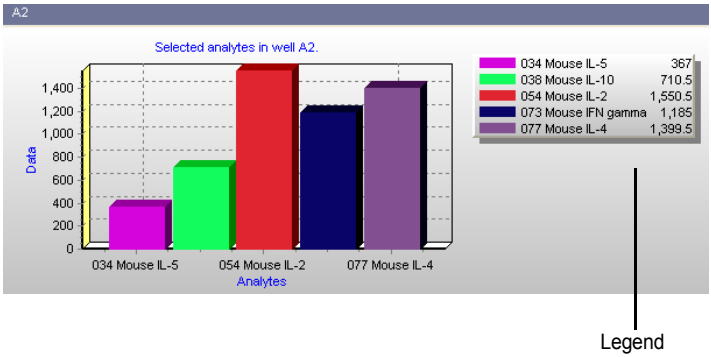


Figure 7.24 Well chart

Settings in the Legends tab determine the location and appearance of a chart legend.

User-Modifiable Legend Settings

Gradient	
Visible	Choose this option to display the legend with a gradient background.
No Middle	
Direction	Make a selection from the drop-down list to set the direction of the gradient displayed in the legend.
Start, Mid, End	Click to open a color palette. Make a selection from the color palette to set the background color for the start, middle, or end portion of the gradient.
Positions	Choose an option to display the legend to the left, right, top, or bottom of the chart.
Shadows	
Color	Click to open a color palette. Make a selection from the color palette to set the color for the shadow at the legend perimeter.
Size	Select a number to set the thickness of the shadow at the perimeter of the legend.
Text style	Make a selection from the drop-down list to select among display options that include order of items, placement of data, hide or unhide data, display percentage values, and display the x-axis value in the legend.
Show Legends	Choose this option to display a legend in a data chart.

Walls Tab

1. To view the chart wall properties, click the Walls tab in the Chart Properties dialog box (Figure 7.25).

   These settings determine the appearance of the left, back, and bottom walls in a three dimensional chart (Figure 7.25).

2. To change a setting, click a wall (Left, Back, or Bottom), and modify the settings.

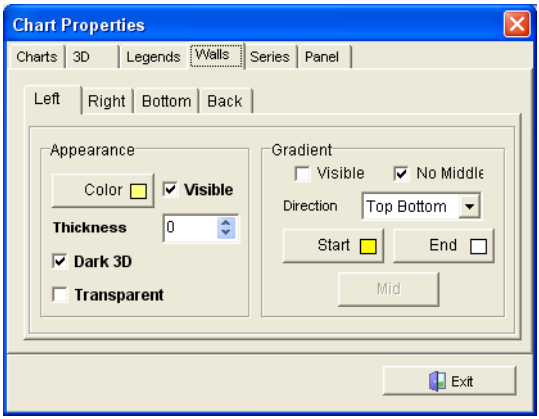


Figure 7.25 Chart Properties dialog box, Walls tab

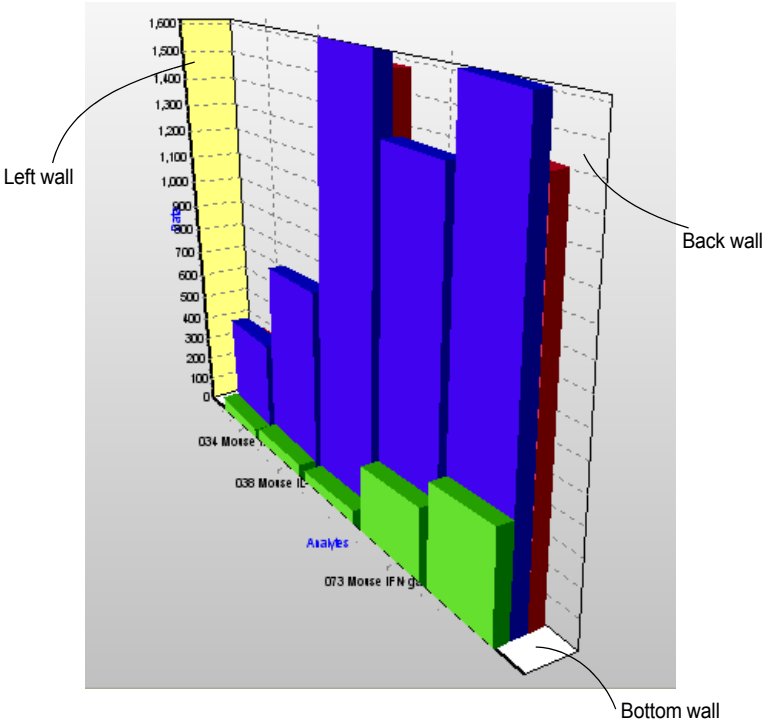


Figure 7.26 3D depth chart

User-Modifiable Wall Settings

Appearance	
Color	Click to open a color palette. Choose a color for the wall from the color palette.
Visible	Choose this option to display the wall color.
Thickness	Change this value to increase or decrease the wall thickness.
Dark 3D	Choose this option to apply a darker color to the inside surface of the wall.
Transparent	Choose an option to display the a transparent wall without color.
Gradient	
Visible	Choose this option to display a gradient wall color.
No Middle	Choose this option if you do not want to display a gradient with only two colors (start and end color). If this option is not chosen, the gradient has three colors (start, middle, and end color).
Direction	Make a selection from the drop-down list to set the direction of the gradient.
Start/Mid/ End	Click to open a color palette. Choose a color for the start, middle, or end of the gradient. Note: If the **No Middle** option is chosen, the **Mid** button is not displayed.

Series Tab

A series is a group of related bars in a chart. For example, in the 3D depth chart, the bars that represent the analytes in a particular well comprise a series (Figure 7.27). In the series tab, you can modify the display of a series.

1. To view the series properties for an open chart(s), click the Series tab in the Chart Properties dialog box (Figure 7.28).
2. To modify the settings for a series, make a selection from the Series drop-down list. If you want to apply the settings to all series in the graph, choose the **Apply to all series** option.

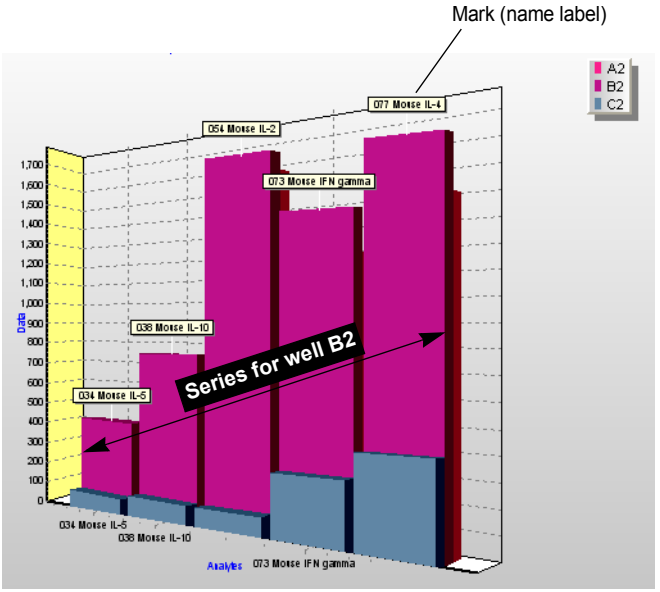


Figure 7.27 3D depth chart

In the 3D depth chart, the bars that represent the analytes in a well are an example of a series.

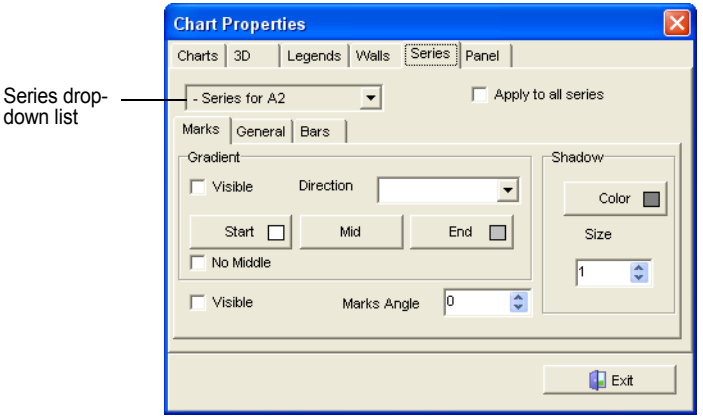


Figure 7.28 Chart Properties dialog box, Series tab, Marks settings

User-Modifiable Series Settings

Marks Settings

These settings determine the display of the name labels (*marks*) in a data chart (Figure 7.27).

Gradient

Visible	Click to display a gradient background in the name label.
Direction	Make a selection from the drop-down list to set the direction of the gradient.
Start/Mid/End	Click to open a color palette. Choose a color for the start, middle, or end of the gradient.
No Middle	Choose this option to display a two color gradient. If this option is not chosen, the gradient has three colors (start, middle, and end color)
Visible	Choose an option to display the name labels for the selected series.
Marks Angle	Click the up or down arrows to set the display angle for the name labels.

Shadow

Color	Click to open a color palette. Choose a color for the shadow of the name label.
Size	Click the up or down arrows to set the thickness of the name label shadow.

General Settings

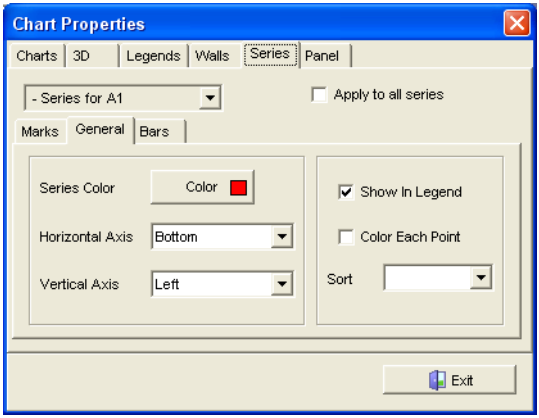


Figure 7.29 Chart Properties dialog box, Series tab, General settings

Series Color	Click to open a color palette. Choose a color for the selected series.
Horizontal Axis	Make a selection from the drop-down list to display the horizontal axis labels at the bottom, top, or bottom and top of the chart.
Vertical Axis	Make a selection from the drop-down list to display the vertical axis labels to the left, right, or left and right of the chart.
Show in Legend	Choose this option to display the series name in the chart legend.
Color Each Point	Choose this option to display each bar in the series in a different color.
Sort	Make a selection from the drop-down list to choose a descending or ascending sort order for the series. Note: After a series is sorted, it cannot be unsorted. To display the series prior to sorting, simply close the chart, then regenerate it.

Bar Settings

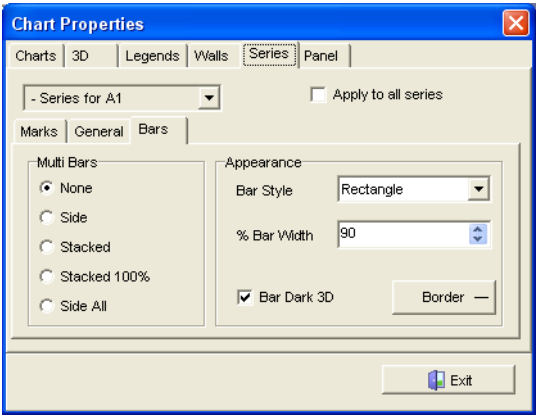


Figure 7.30 Chart Properties dialog box, Series tab, Bar settings

Multi Bars

None Choose this option to display the bars in the default orientation. For example, Figure 7.31 shows the default orientation of the 3D depth chart.

Side Choose this option to display the bars side-by-side along the x-axis (Figure 7.31).

Stacked Choose this option to combine the separate bars of a series into one vertical column (Figure 7.32).

Stacked 100% Choose this option to display the percentage data for a series in one vertical bar. Each segment of the bar represents the percent a particular series value contributes to the total bar value (Figure 7.32).

Side All Choose this option to display all bars of the same type in a side-by-side orientation.

Appearance

Bar Style Make a selection from the drop-down list to choose a bar style.

% Bar Width Click the up or down arrows to change the bar width.

Bar Dark 3D Choose this option to display three dimensional bar demarcations.

Border Click **Border** to open a dialog box of style options for borders of bars.

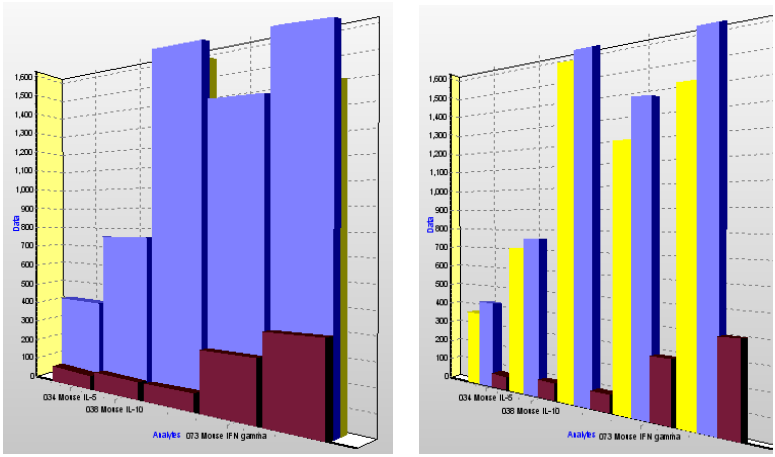


Figure 7.31 3D depth chart
None option is the default display (left). Side option (right).

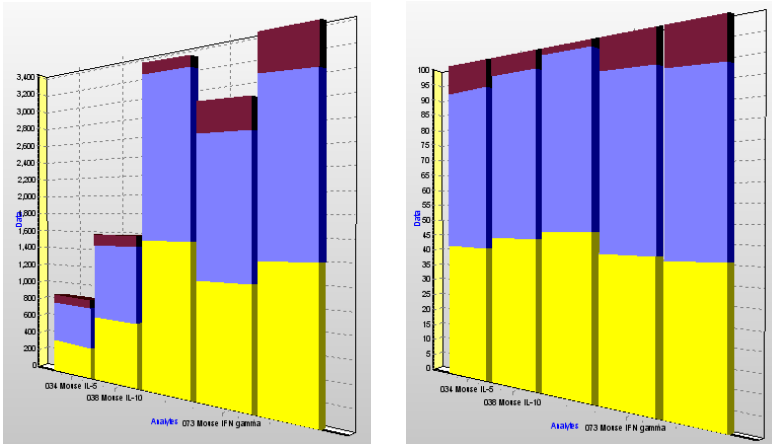


Figure 7.32 3D depth chart
Stacked option (left). Stacked 100% option (right)

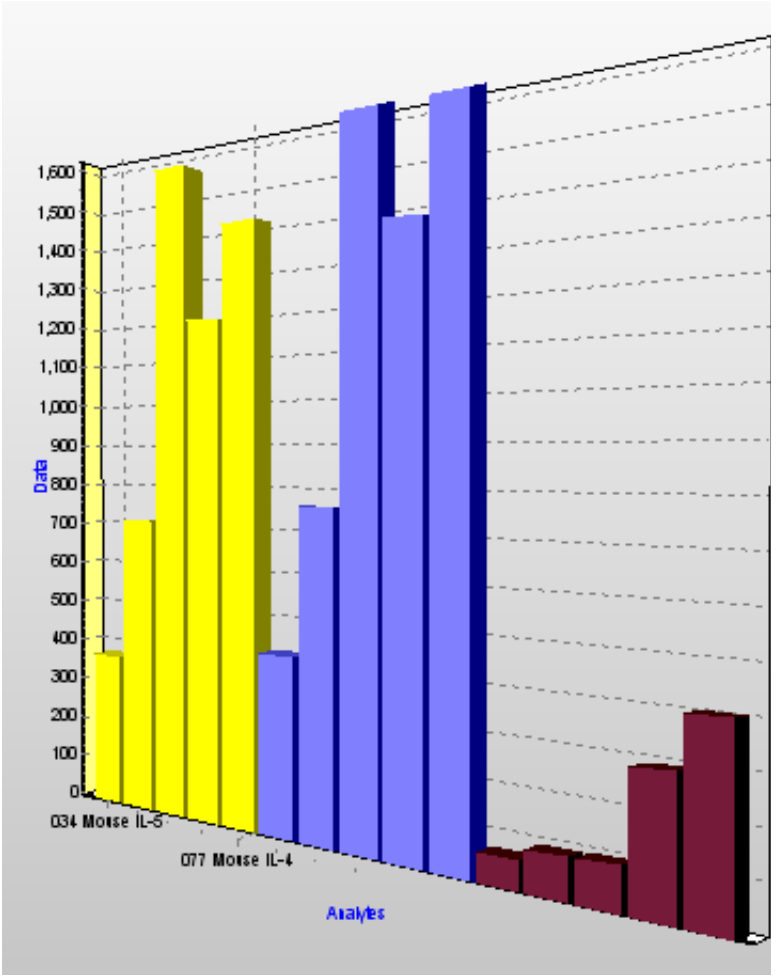


Figure 7.33 3D depth chart
Side All option.

Intensity Map Settings

If an intensity map is open, the Series tab shows Intensity settings (Figure 7.34).

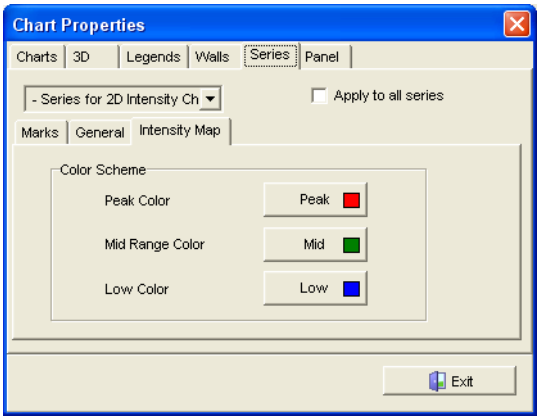


Figure 7.34 Chart Properties dialog box, Series tab
If an intensity map is open in the data chart window, the intensity map color scheme settings are available.

To change a color (peak, mid-range, or low color) in the intensity map:

1. Click the button for the color that you want to change (for example, Peak).

   ⇒ The color palette appears (Figure 7.35).

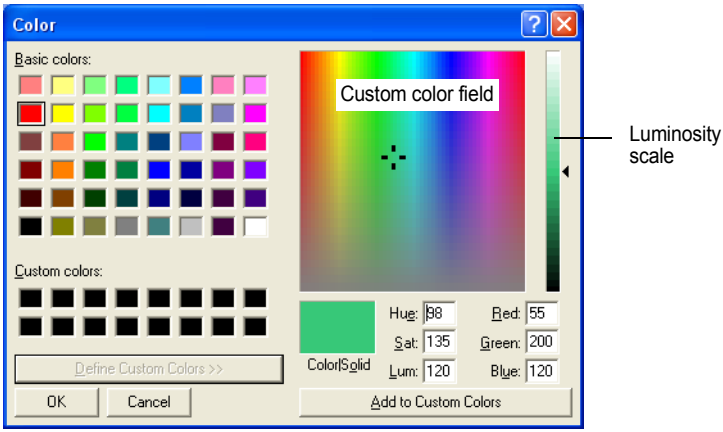


Figure 7.35 Color palette

2. To select a predefined color, click one of the basic colors.
3. To define a custom color:
   a. Click **Define Custom Colors**, then use the click-and-drag method to move the cross hairs in the custom color field.
   b. Adjust the color brightness using the luminosity scale to the right.
   c. When finished, click **Add to Custom Colors** to apply the color.
4. Click **OK** to close the color palette.

Panel Tab

The panel settings determine the appearance of the display area behind the chart.

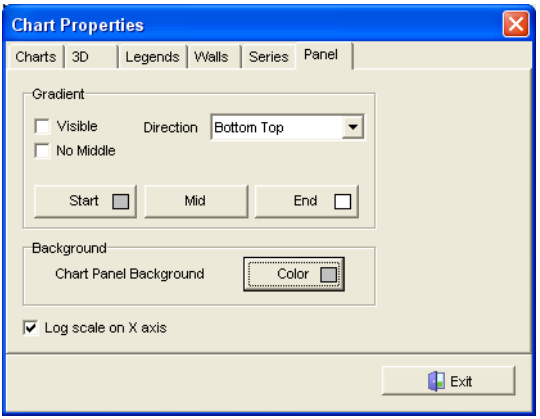


Figure 7.36 Chart properties dialog box, Panel tab

Gradient	
Visible	Choose this option to display a gradient in the area behind the chart.
No Middle	Choose this option to display a two color gradient (start and end color) in the area behind the chart. If you do not choose this option, you can specify three colors for the gradient (start, middle, and end).
Direction	Make a selection from the drop-down list to set the direction of the gradient.
Start/Mid/ End	Click to open a color palette. Choose a color for the start, middle, or end of the gradient.
Background	
Chart Panel Background-Color	Click to open the color palette. Choose a color for the display area behind the chart.
Log scale on X axis	Choose this option to display a log scale on the x-axis for a standard curve only.

NOTE: The Gradient option overrides the Background option.

This chapter explains the types of reports that are available and how to use the report generator. You can preview, print, or save a report.

8.1 Generating a Report

1. Open the plate (.csv or .mlx) for the report.

NOTE: The report generator is only available if a plate window is open and active.

2. Click the **Report Generator** button.
   ⇒ The report generator appears (Figure 8.1).

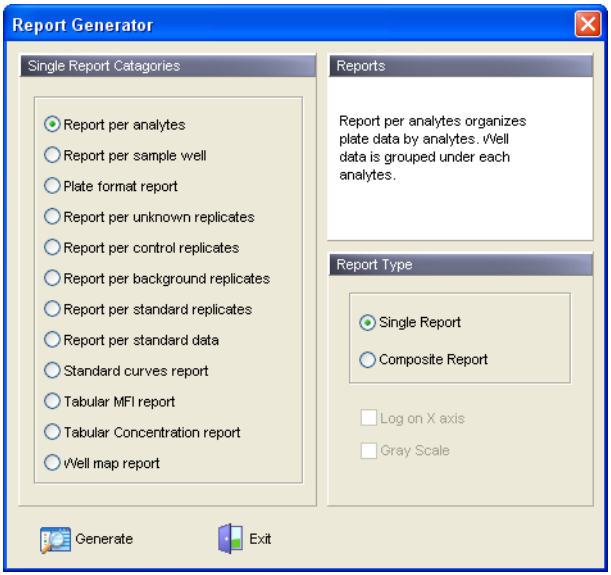


Figure 8.1 Report generator

3. To generate a single report, choose a report category and the **Single Report** option. To generate a composite report, choose the report categories of interest and the **Composite Report** option.

   Table 8.1 lists the available types of reports.

Table 8.1 MasterPlex™ QT reports

Report Name	Contents
Report per analytes	MFI, bead count, and concentration data organized by analyte name (Figure 8.7 on page 8.8)
Report per sample well	MFI, bead count, and concentration data organized by sample well (Figure 8.8 on page 8.9).
Plate format report	Organized by data types (MFI, bead count, or concentration) that are displayed in the well grid format (Figure 8.9 on page 8.10)
Report per unknown replicates	Considers each group of unknown wells a replicate. Includes the mean, standard deviation, and CV% for each unknown group.
Report per control replicates	Considers each group of control wells a replicate. Includes the mean, standard deviation, and CV% for each control group.
Report per background replicates	Includes the mean, standard deviation, and CV% for the background wells.
Report per standard replicates	Considers each group of standard wells a replicate. Includes the mean, standard deviation, and CV% for each standard group (Figure 8.11 on page 8.12).
Report per standard data	Standard data with expected and calculated values, residuals, and per cent recovery (Figure 8.12 on page 8.13).
Standard curves report	Local standard curves generated on the current plate (Figure 8.14 on page 8.15).

Table 8.1 MasterPlex™ QT reports

Report Name	Contents
Tabular MFI report	Displays a table of MFI data per analyte (column) and well (row)
Tabular concentration report	Displays a table of concentration data per analyte (column) and well (row)
Well map report	A layout of the plate that shows the well type, the group number for each well, and the links between standards and unknown groups.

4. To apply a log scale to the x-axis of standard curves in the Standard curves report, choose the **Log on X axis** option.
5. Click **Generate** to display the report.
   ⇒ The report window opens and displays the report. The report name is added to the plate navigator under the report node for the plate.
6. To display a log scale x-axis in the Standard curves report, choose the **Log on X axis** option.
7. To print the Standard curves report on a monochromatic printer, choose the **Gray Scale** option.

8.2
Working With Reports

If the report window is open, the report toolbar is available (Figure 8.2). You can print report or save a report.

Figure 8.2 Report window toolbar

Table 8.2 Report window toolbar button functions

Toolbar Button	Click to...
	Change the magnification in the Report window. (Make a selection from the drop-down list of view options.)
	Display the first page in the report.
	Display the previous page.
	Display the next page.
	Display the last page.
	Open the Save As dialog box.
	Open the Print dialog box (Figure 8.6).

Saving a Report

1. Click the **Export Report** button .

   ⇒ The Save As dialog box appears (Figure 8.3).

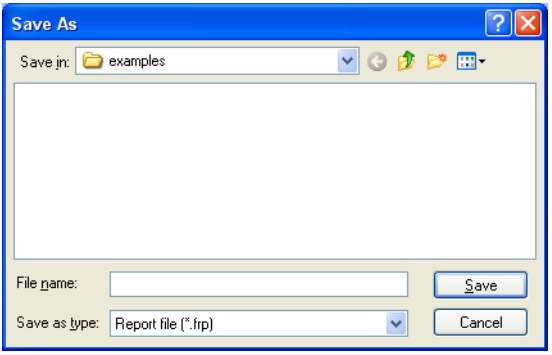


Figure 8.3 Save As dialog box

2. Enter a file name
3. Choose a file type from the **Save as type** drop-down list.

   A report may be saved in .frp, .csv, .txt, .emf, .wmf, .pdf, .bmp, .rtf, or .htm format.

4. Click **Save**.

NOTE: A report saved in the*.frp file format can only be opened in the MasterPlex™ QT application.

Viewing Reports

To view a report generated in the current session, click the report of interest in the Plate Navigator. To view a report saved during a previous session, use the report viewer.

1. To open a saved report, select **File > Report Viewer** from the menu bar.

   ⇒ The Report Viewer appears (Figure 8.4).

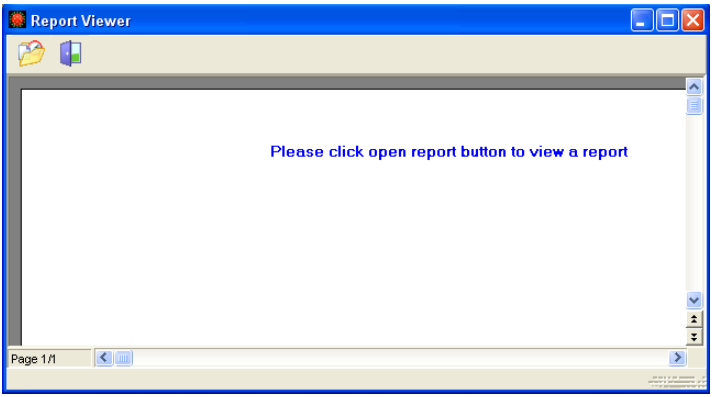


Figure 8.4 Report viewer

2. Click the **Open** button .
   ⇒ The Open dialog box appears (Figure 8.5).

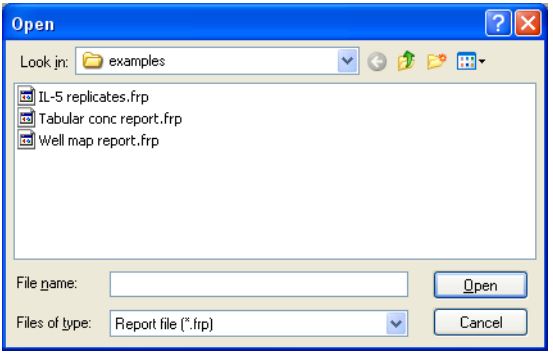


Figure 8.5 Open dialog box

3. Double-click the report that you want to open.
   ⇒ The plate viewer displays the report.
4. To close the report viewer, click the **Close** button .

Printing a Report

1. Double-click the report that you want to print.
2. Click the **Print Report** button .
   ⇒ The Print dialog box appears (Figure 8.6).

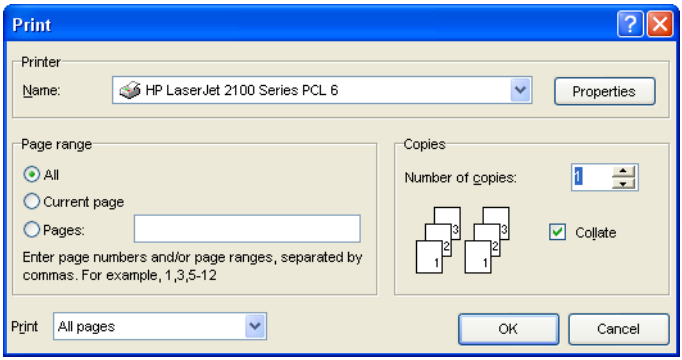


Figure 8.6 Print dialog box

3. Choose the print options of interest and click **OK**.

MasterPlex QT Report By Analyte

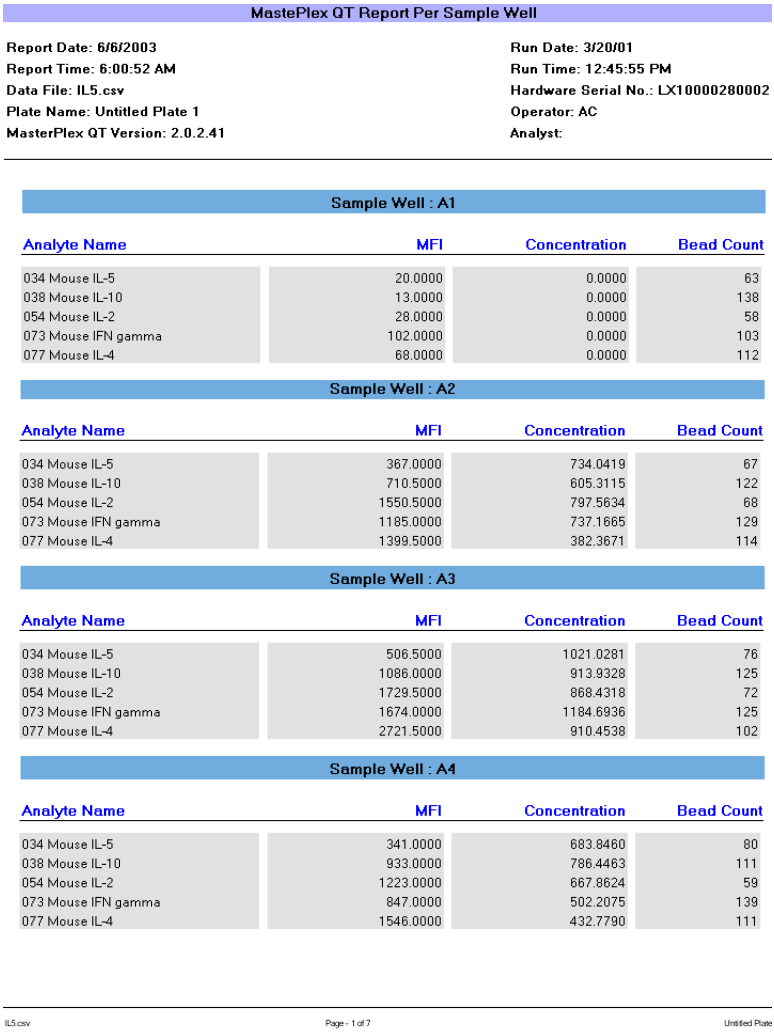
Report Date: 6/6/2003
Report Time: 6:59:58 AM
Data File: IL5.csv
Plate Name: Untitled Plate 1
MasterPlex QT Version: 2.0.2.41

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX1000028000
Operator: AC
Analyst:

Analyte Name : 034 Mouse IL-5

Well	MFI	Concentration	Bead Count
A1	20.0000	0.0000	63
A2	367.0000	734.0419	67
A3	506.5000	1021.0281	76
A4	341.0000	683.8460	80
B1	1308.0000	9320.0673	95
B2	429.0000	857.2340	66
B3	480.5000	964.6140	78
B4	392.0000	783.0479	79
C1	1084.5000	3494.0283	72
C2	82.0000	168.0968	79
C3	88.0000	182.9781	75
C4	78.0000	157.8725	70
D1	525.0000	1062.2069	79
D2	88.5000	184.1962	66
D3	82.0000	168.0968	76
D4	96.5000	203.2842	74
E1	201.0000	418.7686	81
E2	37.5000	14.4666	82
E3	38.0000	18.3328	85
E4	37.0000	9.9752	96
F1	66.5000	126.7273	80
F2	32.0000	MFI<MIN	79
F3	32.0000	MFI<MIN	66
F4	34.0000	MFI<MIN	74
G1	39.0000	25.0488	78
G2	18.0000	MFI<MIN	81
G3	18.0000	MFI<MIN	68
G4	23.0000	MFI<MIN	69
H1	26.5000	MFI<MIN	68
H2	19.0000	MFI<MIN	69
H3	17.5000	MFI<MIN	80
H4	27.0000	MFI<MIN	42

Figure 8.7 Report by analyte
Data are organized by analyte name.



MastePlex QT Report Per Sample Well

Report Date: 6/6/2003
Report Time: 6:00:52 AM
Data File: IL5.csv
Plate Name: Untitled Plate 1
MasterPlex QT Version: 2.0.2.41

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No.: LX10000280002
Operator: AC
Analyst:

Sample Well : A1

Analyte Name	MFI	Concentration	Bead Count
034 Mouse IL-5	20.0000	0.0000	63
038 Mouse IL-10	13.0000	0.0000	138
054 Mouse IL-2	28.0000	0.0000	58
073 Mouse IFN gamma	102.0000	0.0000	103
077 Mouse IL-4	68.0000	0.0000	112

Sample Well : A2

Analyte Name	MFI	Concentration	Bead Count
034 Mouse IL-5	367.0000	734.0419	67
038 Mouse IL-10	710.5000	605.3115	122
054 Mouse IL-2	1550.5000	797.5634	68
073 Mouse IFN gamma	1185.0000	737.1665	129
077 Mouse IL-4	1399.5000	382.3671	114

Sample Well : A3

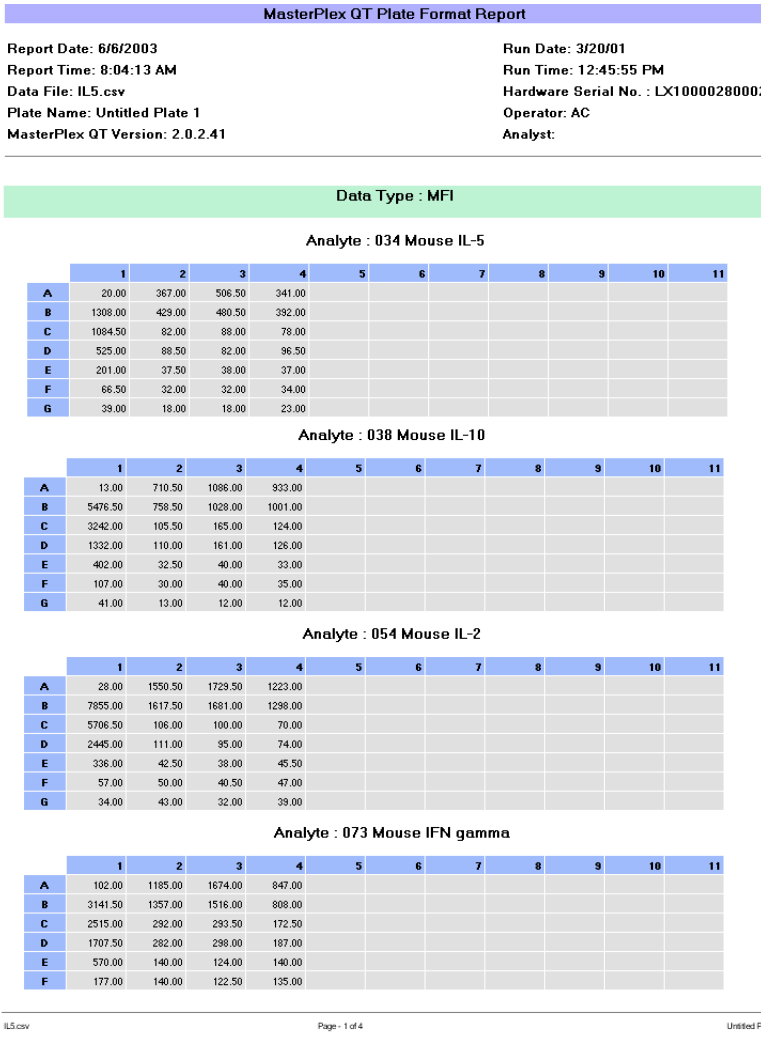
Analyte Name	MFI	Concentration	Bead Count
034 Mouse IL-5	506.5000	1021.0281	76
038 Mouse IL-10	1086.0000	913.9328	125
054 Mouse IL-2	1729.5000	868.4318	72
073 Mouse IFN gamma	1674.0000	1184.6936	125
077 Mouse IL-4	2721.5000	910.4538	102

Sample Well : A4

Analyte Name	MFI	Concentration	Bead Count
034 Mouse IL-5	341.0000	683.8460	80
038 Mouse IL-10	933.0000	786.4463	111
054 Mouse IL-2	1223.0000	667.8624	59
073 Mouse IFN gamma	847.0000	502.2075	139
077 Mouse IL-4	1546.0000	432.7790	111

IL5.csv Page - 1 of 7 Untitled Plate

Figure 8.8 Report per sample well
Data are organized by well location.



MasterPlex QT Plate Format Report

Report Date: 6/6/2003
Report Time: 8:04:13 AM
Data File: IL5.csv
Plate Name: Untitled Plate 1
MasterPlex QT Version: 2.0.2.41

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX1000028000
Operator: AC
Analyst:

Data Type : MFI

Analyte : 034 Mouse IL-5

	1	2	3	4	5	6	7	8	9	10	11
A	20.00	367.00	506.50	341.00							
B	1308.00	429.00	480.50	392.00							
C	1084.50	82.00	88.00	78.00							
D	525.00	88.50	82.00	96.50							
E	201.00	37.50	38.00	37.00							
F	66.50	32.00	32.00	34.00							
G	39.00	18.00	18.00	23.00							

Analyte : 038 Mouse IL-10

	1	2	3	4	5	6	7	8	9	10	11
A	13.00	710.50	1086.00	933.00							
B	5476.50	758.50	1028.00	1001.00							
C	3242.00	105.50	165.00	124.00							
D	1332.00	110.00	161.00	126.00							
E	402.00	32.50	40.00	33.00							
F	107.00	30.00	40.00	35.00							
G	41.00	13.00	12.00	12.00							

Analyte : 054 Mouse IL-2

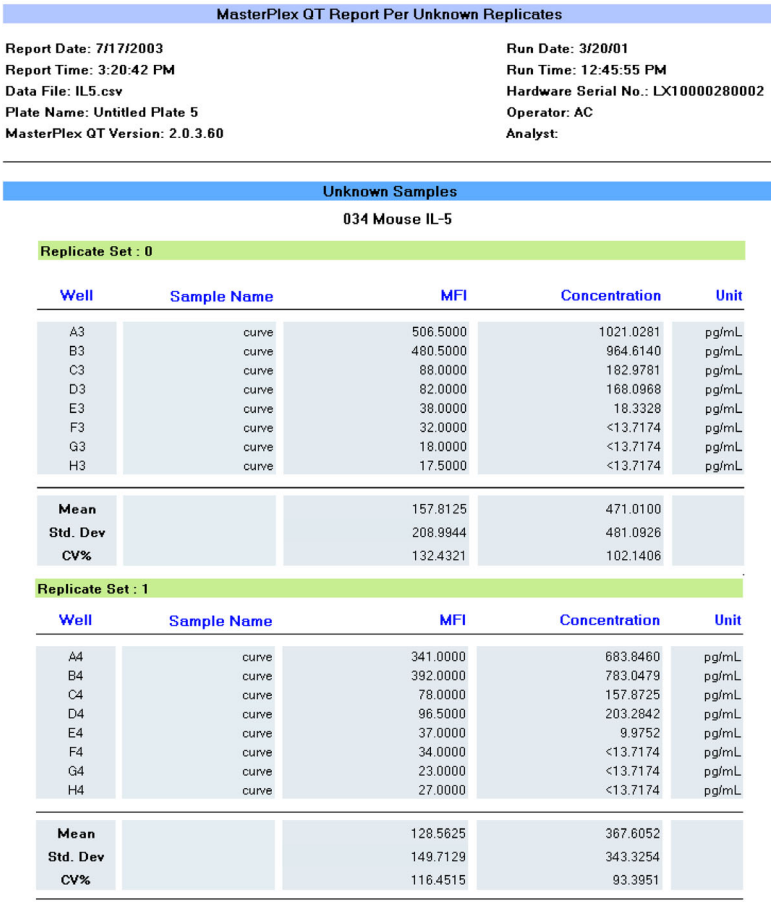
	1	2	3	4	5	6	7	8	9	10	11
A	28.00	1550.50	1729.50	1223.00							
B	7855.00	1617.50	1681.00	1298.00							
C	5706.50	106.00	100.00	70.00							
D	2445.00	111.00	95.00	74.00							
E	336.00	42.50	38.00	45.50							
F	57.00	50.00	40.50	47.00							
G	34.00	43.00	32.00	39.00							

Analyte : 073 Mouse IFN gamma

	1	2	3	4	5	6	7	8	9	10	11
A	102.00	1185.00	1674.00	847.00							
B	3141.50	1357.00	1516.00	808.00							
C	2515.00	292.00	293.50	172.50							
D	1707.50	282.00	298.00	187.00							
E	570.00	140.00	124.00	140.00							
F	177.00	140.00	122.50	135.00							

IL5.csv Page - 1 of 4 Untitled F

Figure 8.9 Plate format report
Each data type is displayed in a well grid format.



MasterPlex QT Report Per Unknown Replicates

Report Date: 7/17/2003
Report Time: 3:20:42 PM
Data File: IL5.csv
Plate Name: Untitled Plate 5
MasterPlex QT Version: 2.0.3.60

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No.: LX10000280002
Operator: AC
Analyst:

Unknown Samples

034 Mouse IL-5

Replicate Set : 0

Well	Sample Name	MFI	Concentration	Unit
A3	curve	506.5000	1021.0281	pg/mL
B3	curve	480.5000	964.6140	pg/mL
C3	curve	88.0000	182.9781	pg/mL
D3	curve	82.0000	168.0968	pg/mL
E3	curve	38.0000	18.3328	pg/mL
F3	curve	32.0000	<13.7174	pg/mL
G3	curve	18.0000	<13.7174	pg/mL
H3	curve	17.5000	<13.7174	pg/mL
Mean		157.8125	471.0100	
Std. Dev		208.9944	481.0926	
CV%		132.4321	102.1406	

Replicate Set : 1

Well	Sample Name	MFI	Concentration	Unit
A4	curve	341.0000	683.8460	pg/mL
B4	curve	392.0000	783.0479	pg/mL
C4	curve	78.0000	157.8725	pg/mL
D4	curve	96.5000	203.2842	pg/mL
E4	curve	37.0000	9.9752	pg/mL
F4	curve	34.0000	<13.7174	pg/mL
G4	curve	23.0000	<13.7174	pg/mL
H4	curve	27.0000	<13.7174	pg/mL
Mean		128.5625	367.6052	
Std. Dev		149.7129	343.3254	
CV%		116.4515	93.3951	

Figure 8.10 Report per unknown replicates

MasterPlex QT Report Per Replicates Controls

Report Date: 7/17/2003
Report Time: 3:41:49 PM
Data File: AverageStd.csv
Plate Name: Untitled Plate 7
MasterPlex QT Version: 2.0.3.60

Run Date: 5/2/2001
Run Time: 3:10:40 PM
Hardware Serial No.: LX10000175005
Operator: training
Analyst:

Control Samples

IL-10

Replicate Set : 0

Well	Sample Name	MFI	Concentration	Unit
A2		8070.0000	8737.8067	pg/mL
B2		6523.0000	3555.0841	pg/mL
C2		3803.0000	1169.1345	pg/mL
D2		1756.0000	425.0784	pg/mL
E2		690.0000	153.6266	pg/mL
F2		279.5000	58.3281	pg/mL
G2		85.5000	11.9726	pg/mL
H2		52.0000	3.1049	pg/mL
Mean		2657.3750	1764.2670	
Std. Dev		3146.2421	3062.7137	
CV%		118.3966	173.5970	

Replicate Set : 1

Well	Sample Name	MFI	Concentration	Unit
A3		8132.0000	9187.8089	pg/mL
B3		6380.5000	3332.3044	pg/mL
C3		3641.0000	1093.5416	pg/mL
D3		1683.5000	404.9261	pg/mL
E3		550.5000	121.0502	pg/mL
F3		198.0000	39.2619	pg/mL
G3		101.5000	15.9976	pg/mL
H3		51.0000	2.8212	pg/mL
Mean		2592.2500	1774.7140	
Std. Dev		3151.6058	3200.4344	
CV%		121.5780	180.3352	

Figure 8.11 Report per control replicates

MasterPlex QT Standard Data Report

Report Date: 7/17/2003	Run Date: 5/2/2001
Report Time: 3:54:00 PM	Run Time: 3:10:40 PM
Data File: AverageStd.csv	Hardware Serial No.: LX10000175005
Plate Name: Untitled Plate 7	Operator: training
MasterPlex QT Version: 2.0.3.60	Analyst:

Analyte : IL-10

Standard Data Set : 0
Model: Four Parameter Logistics

Replicates Set: 0

Well	Sample Name	MFI	Concentration	Unit
A1		8195	9689.2410	pg/mL
Mean		8195.0000	9689.2410	
Std. Dev		8195.0000	9689.2410	
CV%		100.0000	100.0000	

Replicates Set: 1

Well	Sample Name	MFI	Concentration	Unit
B1		6480	3486.5147	pg/mL
Mean		6480.5000	3486.5147	
Std. Dev		6480.5000	3486.5147	
CV%		100.0000	100.0000	

Replicates Set: 2

Well	Sample Name	MFI	Concentration	Unit
C1		3541	1048.7525	pg/mL
Mean		3541.0000	1048.7525	
Std. Dev		3541.0000	1048.7525	
CV%		100.0000	100.0000	

Replicates Set: 3

Well	Sample Name	MFI	Concentration	Unit
D1		1674	402.1697	pg/mL
Mean		1673.5000	402.1697	
Std. Dev		1673.5000	402.1697	
CV%		100.0000	100.0000	

Figure 8.12 Report per standard replicates
This partial view of the report shows data for the first three IL-10 standard replicates.

MasterPlex QT Standard Data Report

Report Date: 7/17/2003	Run Date: 3/20/01
Report Time: 4:29:22 PM	Run Time: 12:45:55 PM
Data File: IL5.csv	Hardware Serial No.: LX10000280002
Plate Name: C:\Program	Operator: AC
MasterPlex QT Version: 2.0.3.60	Analyst:

Analyte : 034 Mouse IL-5

Standard Data Set: 0
Equation Model: Four Parameter Logistics

Well	Sample Name	MFI	Calculated	Expected	Unit	Residuals	%Recovery
B1	curve	1308.0000	9320.0673	10000.0000	pg/mL	679.9327	93.201
C1	curve	1084.5000	3494.0283	3333.3333	pg/mL	160.6950	104.821
D1	curve	525.0000	1062.2069	1111.1111	pg/mL	48.9042	95.599
E1	curve	201.0000	418.7686	370.3704	pg/mL	48.3982	113.068
F1	curve	66.5000	126.7273	123.4568	pg/mL	3.2705	102.649
G1	curve	39.0000	25.0488	41.1523	pg/mL	16.1034	60.869
H1	curve	26.5000	<13.7174	13.7174	pg/mL	13.7174	0.000

Analyte : 038 Mouse IL-10

Standard Data Set: 0
Equation Model: Four Parameter Logistics

Well	Sample Name	MFI	Calculated	Expected	Unit	Residuals	%Recovery
B1	curve	5476.5000	10009.7150	10000.0000	pg/mL	9.7150	100.097
C1	curve	3242.0000	3322.3144	3333.3333	pg/mL	11.0189	99.669
D1	curve	1332.0000	1125.7177	1111.1111	pg/mL	14.6066	101.315
E1	curve	402.0000	357.8735	370.3704	pg/mL	12.4968	96.626
F1	curve	107.0000	111.1588	123.4568	pg/mL	12.2980	90.039
G1	curve	41.0000	48.2824	41.1523	pg/mL	7.1302	117.326
H1	curve	21.0000	26.9207	13.7174	pg/mL	13.2033	196.252

Figure 8.13 Report per standard data

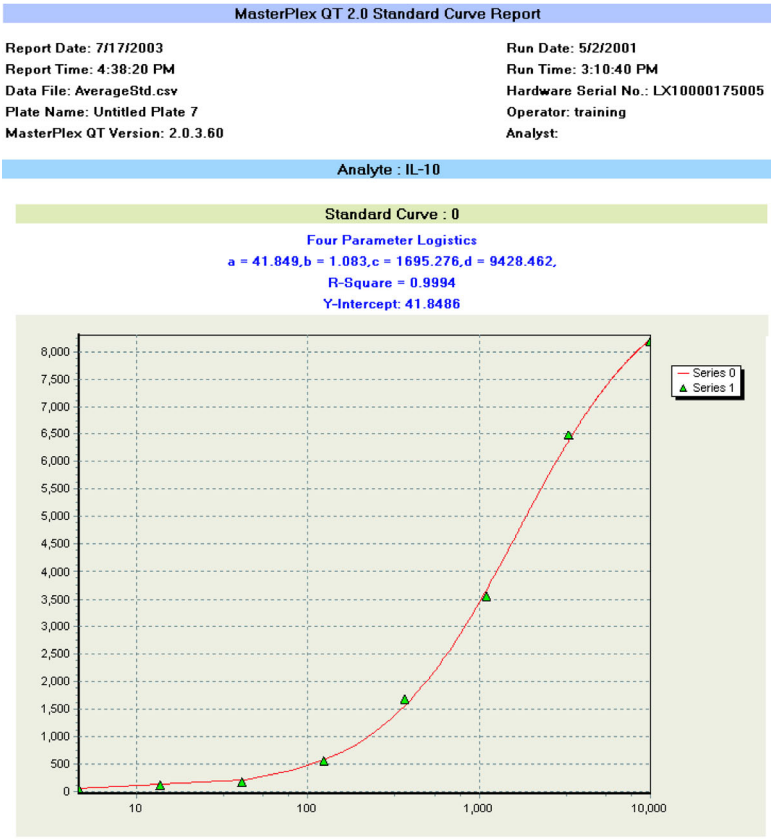


Figure 8.14 Standard curves report
Shows the standard curve(s) for each analyte.

MasterPlex QT Tabular Report

Report Date: 7/17/2003
Report Time: 4:46:46 PM
Data File: IL5.csv
Plate Name: Untitled Plate 5
MasterPlex QT Version: 2.0.3.60

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX10000280002
Operator: AC
Analyst:

Well	Sample Name	034 Mouse IL-5	038 Mouse IL-10	054 Mouse IL-2	073 Mouse IFN	077 Mouse IL-
A1	curve	20.00	13.00	28.00	102.00	68.(
A2	Ind mouse serum	367.00	710.50	1550.50	1185.00	1399.5
A3	90-10 buffer	506.50	1086.00	1729.50	1674.00	2721.5
A4	Sigma pooled	341.00	933.00	1223.00	847.00	1546.(
B1	2	1308.00	5476.50	7855.00	3141.50	7792.(
B2	10	429.00	758.50	1617.50	1357.00	1622.(
B3		480.50	1028.00	1681.00	1516.00	2577.(
B4		392.00	1001.00	1298.00	808.00	1672.(
C1	3	1084.50	3242.00	5706.50	2515.00	5772.(
C2		82.00	105.50	106.00	292.00	400.(
C3		88.00	165.00	100.00	293.50	785.(
C4		78.00	124.00	70.00	172.50	430.(
D1	4	525.00	1332.00	2445.00	1707.50	2980.(
D2		88.50	110.00	111.00	282.00	389.5
D3		82.00	161.00	95.00	298.00	849.(
D4		96.50	126.00	74.00	187.00	430.(
E1	5	201.00	402.00	336.00	570.00	1474.5
E2		37.50	32.50	42.50	140.00	158.(
E3		38.00	40.00	38.00	124.00	259.5
E4		37.00	33.00	45.50	140.00	158.(
F1	6	66.50	107.00	57.00	177.00	584.(
F2		32.00	30.00	50.00	140.00	163.(
F3		32.00	40.00	40.50	122.50	272.(
F4		34.00	35.00	47.00	135.00	161.(
G1	7	39.00	41.00	34.00	125.50	265.(
G2		18.00	13.00	43.00	101.00	74.5
G3		18.00	12.00	32.00	109.00	69.(
G4		23.00	12.00	39.00	125.00	67.(

Figure 8.15 Tabular MFI report

MasterPlex QT Tabular Report

Report Date: 7/17/2003
Report Time: 4:51:59 PM
Data File: IL5.csv
Plate Name: Untitled Plate 5
MasterPlex QT Version: 2.0.3.60

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX10000280002
Operator: AC
Analyst:

Well	Sample Name	034 Mouse IL-5	038 Mouse IL-10	054 Mouse IL-2	073 Mouse IFN	077 Mouse IL-
A1	curve	<13.72	17.62	89.09	40.27	<13.
A2	Ind mouse serum	734.04	605.31	797.56	737.17	382.
A3	90-10 buffer	1021.03	913.93	868.43	1184.69	910.
A4	Sigma pooled	683.85	786.45	667.86	502.21	432.
B1	2	9320.07	10009.72	10288.67	13385.94	9808.
B2	10	857.23	644.05	824.06	875.96	459.
B3		964.61	865.27	849.20	1020.62	843.
B4		783.05	842.76	697.68	477.44	477.
C1	3	3494.03	3322.31	3258.24	2865.21	3464.
C2		168.10	109.80	150.22	169.07	77.
C3		182.98	162.27	146.03	169.98	187.
C4		157.87	126.41	124.02	93.09	85.
D1	4	1062.21	1125.72	1159.72	1222.64	1037.
D2		184.20	113.87	153.67	163.00	74.
D3		168.10	158.82	142.49	172.71	206.
D4		203.28	128.18	127.07	102.86	85.
E1	5	418.77	357.87	284.09	332.92	407.
E2		14.47	39.44	101.86	70.16	10.
E3		18.33	47.26	98.00	58.13	38.
E4		9.98	39.97	104.40	70.16	10.
F1	6	126.73	111.16	113.83	96.15	129.
F2		<13.72	36.78	108.14	70.16	12.
F3		<13.72	47.26	100.15	56.96	41.
F4		<13.72	42.07	105.66	66.46	11.
G1	7	25.05	48.28	94.49	59.28	40.
G2		<13.72	17.62	102.29	39.41	<13.
G3		<13.72	16.41	92.70	46.17	<13.
G4		<13.72	16.41	98.86	58.90	<13.

Figure 8.16 Tabular concentration report

MasterPlex QT Well Map Report

Report Date: 7/17/2003
Report Time: 5:02:01 PM
Data File: IL5.csv
Plate Name: C:\Program
MasterPlex QT Version: 2.0.3.60

Run Date: 3/20/01
Run Time: 12:45:55 PM
Hardware Serial No. : LX10000280002
Operator: AC
Analyst:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bkg-0	Unk-0	Unk-1	Unk-2								
B	Std-0	Unk-0	Unk-1	Unk-2								
C	Std-0	Unk-0	Unk-1	Unk-2								
D	Std-0	Unk-0	Unk-1	Unk-2								
E	Std-0	Unk-0	Unk-1	Unk-2								
F	Std-0	Unk-0	Unk-1	Unk-2								
G	Std-0	Unk-0	Unk-1	Unk-2								

Figure 8.17 Well map report

Some of the software settings can be modified. This appendix explains the preferences (user-modifiable software settings) that are available to you. These settings are displayed in the Preferences dialog box.

To open the Preferences dialog box (Figure A.1), click the **Preferences** button . Alternatively, select **File** > **Preferences** from the menu bar.

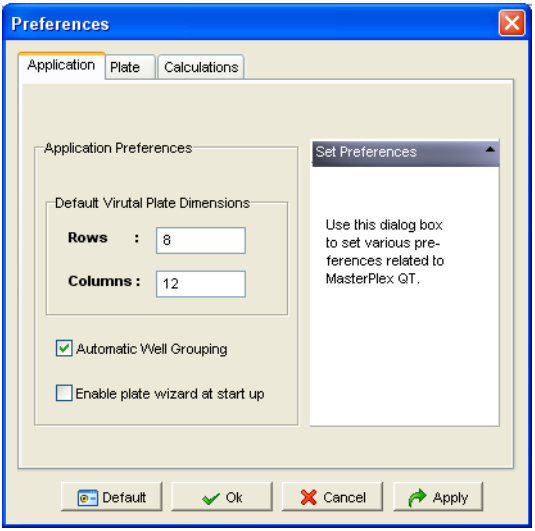


Figure A.1 Preferences dialog box
Application tab default settings

A.1 Application Tab

The application preferences (Figure A.1) specify:

- the number of rows and columns for a virtual plate displayed in displayed in the plate wizard
- whether the plate wizard appears when the MasterPlex™ QT software starts

1. To change the row and column dimensions of the well grid, enter the number of rows and columns for the well grid.

2. If you do not want to display the plate wizard at program start up, remove the check mark from the **Enable plate wizard at start up** option.
3. Click **Apply** when you are finished.
4. To return the application preferences to the factory set defaults, click **Default**.

A.2
Plate Tab

The Plate preferences specify:

- how to compute background and when to subtract background
- threshold values for concentration, MFI, and bead count
- the plate and analyst name

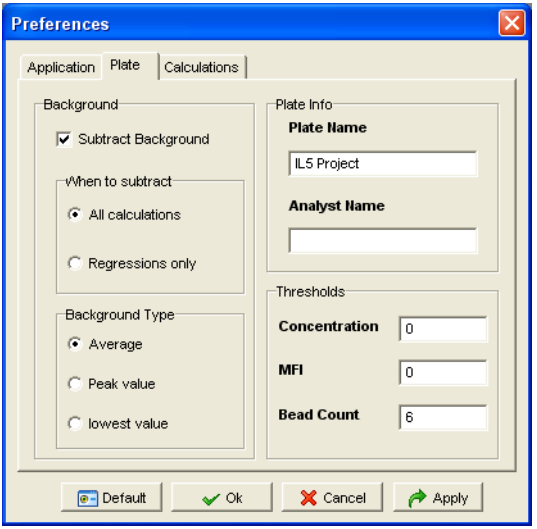


Figure A.2 Preferences dialog box
Plate tab default settings

NOTE: If a plate (.csv or .mlx) is not open, the Preferences dialog box does not display the Plate tab.

Background Options

You can specify whether you want to consider background data in the calculation of analyte concentrations. There are two methods of computing background-subtracted analyte concentrations: All Calculations or Regressions Only.

Subtract Background	Choose this option if you want to compute background-subtracted analyte concentrations. If this option is not chosen, the background MFI value is not considered during calculation of the analyte concentrations.
All Calculations	This method of computing background-subtracted analyte concentrations subtracts the background MFI from each member of the standard data set, then fits the standard curve. The method subtracts background MFI from unknown MFI, then interpolates the unknown analyte concentration from the standard curve.
Regressions Only	This method of computing background-subtracted analyte concentrations subtracts the background MFI from each member of the standard data set, then fits the standard curve. The method does not subtract the background MFI from the unknown MFI before interpolating the unknown analyte concentration.

NOTE: The **All Calculations** method is recommended. The **Regressions Only** method provides backward compatibility with data generated in MasterPlex™ QT 1.0.

Background Type

If there are three or more background wells in the assay, choose one of the following methods for computing background MFI.

Average	Background (Bkg) MFI = (Bkg MFI_1 + Bkg MFI_2 +... Bkg MFI_n)/n where n = the number of background wells in the plate
Peak Value	Highest background MFI value.
Lowest Value	Lowest background MFI value.

Plate Info

Plate Name	Displays the name assigned to the result file in the Luminex™ 100 software. If you want to edit the plate name, enter a new name.
Analyst Name	Displays the analyst name entered in the Luminex™ 100 software. If you want to edit the analyst name, enter a new name.

Thresholds

You can enter an MFI, count, or concentration threshold for a plate. The software can identify wells that contain data less than the user-specified threshold.

To set a threshold(s):

1. Enter the MFI, count, or concentration threshold in the Preferences dialog box (Figure A.2).
2. Click **Apply** when you are finished.
3. To return the plate preferences to the factory set defaults, click **Default**.

To identify the wells in the Plate window that contain data less than threshold:

1. Make a selection from the analyte panel.
2. In the Plate window, select the data type (MFI, count, or concentration) from the data-type drop-down list.
3. To identify wells with MFI data less than threshold, click the button.

⇒ A red border is placed around the well (Figure A.3).

To identify wells with bead count data less than threshold, click the button.

⇒ A red border is placed around the well.

To identify wells with concentrations less than threshold, click the button.

⇒ A red border is placed around the well.

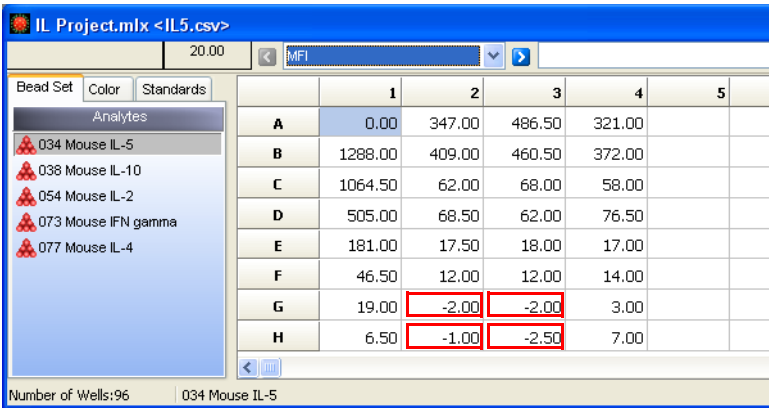


Figure A.3 Plate window

Red border identifies wells with a MFI value less than the user-specified threshold for the selected analyte.

A.3
Calculations Tab

The Calculations preferences (Figure A.4) specify how to:

- fit the standard curve when there are replicate standard data sets
- display concentrations for diluted unknowns that were diluted (the diluted concentration or the original undiluted concentration)

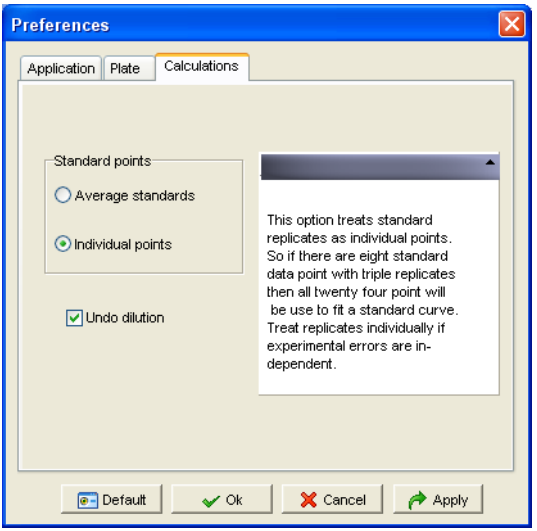

Preferences
Application
Plate
Calculations
Standard points
Average standards
Individual points
Undo dilution
This option treats standard replicates as individual points. So if there are eight standard data point with triple replicates then all twenty four point will be use to fit a standard curve. Treat replicates individually if experimental errors are independent.
Default
Ok
Cancel
Apply

Figure A.4 Preferences dialog box, Calculations tab

Standard Points

If the plate contains replicate standard data sets, there are two ways to fit a standard curve.

Average standards	The replicate standard data points are averaged and the standard curve is fitted to the single set of averaged data. Choose this option if the experimental errors are not independent of each other
Individual points	Replicate standard data points are not averaged and the standard curve is fitted to all of the data points. For example, if there are three replicates of eight standard wells, the standard curve is fitted using all 24 data points. Choose this option if experimental errors are independent of each other.

Diluted Unknowns

Samples can be diluted prior to the assay and analysis. After MasterPlex QT interpolates the diluted unknown analyte concentrations from the standard curve, it can compute and display the original, undiluted concentration in the Plate window.

Original concentration = Diluted concentration * Dilution Factor

The dilutions factors are manually entered in the Plate window. (For more information see

4. Click **Apply** when you are finished.

To return the plate preferences to the factory set defaults, click **Default**.

The toolbars that are available depend on the types of windows that are open in the main display area.

B.1 Main Toolbar

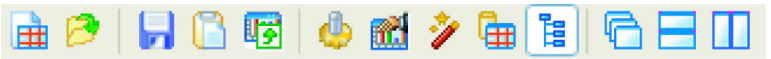


Figure B.1 Main toolbar

Table B.1 Main toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
File → New Plate		Displays the Open dialog box so that a Luminex results file (.csv) may be imported.
File → Open Plate		Displays the Open dialog box so that a MasterPlex QT file (.mlx) may be opened.
File → Save		Displays the Save As dialog box so that a results file (.csv) may be saved in MasterPlex QT file format (.mlx).
Edit → Copy		Copies the data in the active window to the system clipboard.
Edit → Export Chart		Displays the Export Chart dialog box.
File → Preferences		Displays the Preferences dialog box.
Charts → Chart Properties		Displays the Chart Properties dialog box.
		Displays the Plate Wizard.
Plate → Template Manager		Opens the Template Manager.
Show→Show/Hide→Navigator		Shows or hides the Plate Navigator.

Table B.1 Main toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
Window → Cascade		Arranges the open windows in a cascade in the main display area.
Window → Tile Horizontally		Tiles the open windows horizontally in the main display area.
Window → Tile Vertically		Tiles the open windows vertically in the main display area.

B.2
Template Toolbar

The template toolbar is available when a Plate window is open. To show or hide the plate toolbar, select **Show > Show/Hide > Plate Tools** from the menu bar.

Figure B.2 Template toolbar

Table B.2 Template toolbar buttons and functions

Menu Bar Command	Template Toolbar Button	Function
Template → Mark Wells → Mark Standard Well		Defines a well(s) selected in the Plate window as a member(s) of a standard data set.
Template → Mark Wells → Mark Unknown Well		Defines a well(s) selected in the Plate window as an unknown well.
Template → Mark Wells → Mark Control Well		Defines a well(s) selected in the Plate window as a control well.
Template → Mark Wells → Mark Background Well		Defines a well(s) selected in the Plate window as a background well.

Table B.2 Template toolbar buttons and functions

Menu Bar Command	Template Toolbar Button	Function
Template → Unmark Wells		Removes the well type and group designation (if applicable) from a selected well(s).
Template → Mark Wells → Mark Regression Wells		Defines a well(s) selected in the Plate window as a member(s) of a regression analysis data set.
Template → Group Wells		Places selected wells into a group that is identified by a black border around the wells.
Template → Ungroup Wells		Removes a user-selected well(s) from a group.
Template → Autofill		Opens the Autofill dialog box.
Template → Link Standard		Associates well groups with the standard that will be used to compute (interpolate or extrapolate) the analyte concentrations.

B.3
Plate Toolbar

The Plate toolbar is available when a Plate window is open. To show or hide the plate toolbar, select **Show** > **Show/Hide** > **Plate Tools** from the menu bar.

Figure B.3 Plate toolbar

Table B.3 Plate toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
Plate → Selection Tool		Click this button to enable users to select wells in the Plate window using the mouse cursor.
Plate → Edit Wells		Click this button to enable users to edit user-modifiable data in the Plate window.
Plate → Virtual Pipette		Enables the virtual pipette that is used to transfer analyte data from a source plate to a virtual plate.
Plate → Analytes Filter		Opens the virtual analyte filter. Note: This button is only available when the virtual pipette is turned on.
Plate → Import Standards		When toggled on, this button enables the user to copy a standard data set from one plate to another using a drag-and-drop operation.
Plate → Data Charts		Opens a Data Chart window and displays the well chart of the selected data type for the selected well(s).
Plate → View Curves		Opens the Standard Curves window for the active Plate window.
		Displays the Report Generator dialog box.

Table B.3 Plate toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
Show → Show Well Types		Displays a well grid using colors to denote the different well types. Blue = standard well, green = unknown well gray = background well, orange = control well.
Show → Show Well Groups		In the well grid, displays a border around the wells in a group.
Plate → Show MFI < Limit		Displays a red border around wells that contain MFI data less than the user-specified threshold.
Plate → Show CON < Limit		Displays a red border around wells that contain MFI data less than the user-specified concentration threshold.
Plate → Show Count < Limit		Displays a red border around wells that contain count data less than the user-specified count threshold.
Plate → Subtract Background		Displays background-subtracted data in the Plate window.
		Displays the Print Settings dialog box.
Plate → Print Plate		Displays a print preview of the well grid for the selected analyte in the active plate window.

B.4
Calculation Toolbar

The calculation toolbar is available when a Plate window is open. To show or hide the calculation toolbar, select **Show** > **Show/Hide** > **Calculation Tools** from the menu bar.

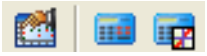


Figure B.4 Calculation toolbar

Table B.4 Calculation toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
Calculation → Model Equations		Displays the Model Equations dialog box so that you can select a model for a standard or regression analysis data set.
Calculate → Calculate STD Curves		Generates the standard curves for the selected standard data set using the selected model and interpolates analyte concentrations from the standard curves.
Calculate → Fit Regression Curves		Generates a Dose-Reponse curve for the selected regression analysis data set using the selected model and determines LogEC50.

B.5
Chart Toolbar

The chart toolbar is available when a Data Chart or Standard Curves window is open. To show or hide the chart toolbar, select **Show** > **Show/Hide** > **Chart Tools** from the menu bar.

Figure B.5 Chart toolbar

Table B.5 Chart toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
Charts → Select Charts		Displays a drop-down list of chart types.
Charts → Chart Select Tool		Click this button to put the Standard Curves window in selection mode. Then press and hold the **Ctrl** key while you click a point(s) in the standard curve to make it an outlier.
Charts → Chart 3D		Toggles the display of a chart between a two or three dimensional view.
Charts → Rotate Chart		Enables you to rotate a chart using a click-and-drag operation.
Charts → Zoom Chart		Enables you to magnify a user-selected area in a chart using a click-and-drag operation.
		Displays or hides the analyte panel in the Data Chart window.
		Displays or hides the scale bar in the Data Chart window.
Charts → Print Chart		Opens the Chart Print Preview dialog box.

B.6
Report Toolbar

The report toolbar is available when the Report window is open. To show or hide the report toolbar, select **Show > Show/Hide > Report Tools** from the menu bar.

Figure B.6 Report toolbar

Table B.6 Report toolbar buttons and functions

Menu Bar Command	Main Toolbar Button	Function
		Displays a drop-down list of magnification options for viewing the report in the Report window.
		Displays the first page in the report.
		Displays the previous page in the report.
		Displays the next page in the report.
		Displays the last page in the report.
		Displays the Save As dialog box so that you may save a report (.frp).
		Displays the Print dialog box so that you may print a report.

This appendix provides background on the four and five parameter logistic curves. It also explains how weighting methods can improve the fit of nonlinear models to data with nonconstant variability (heteroscedasticity).

C.1 Four Parameter Logistic Curve

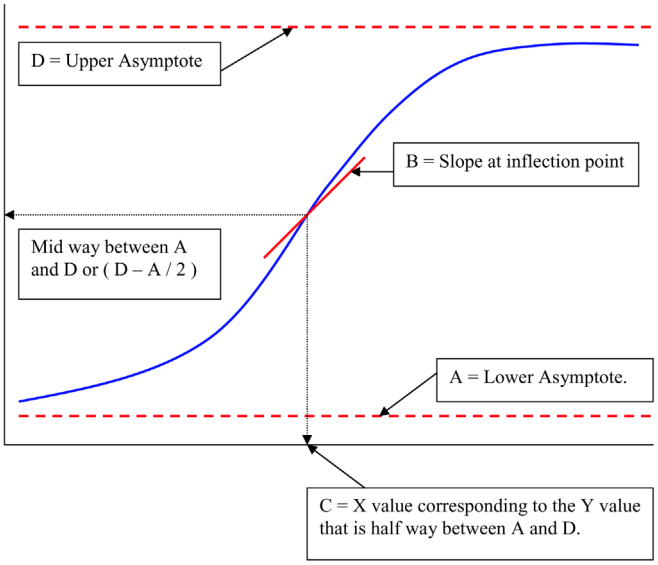

D = Upper Asymptote
B = Slope at inflection point
Mid way between A and D or (D – A / 2)
A = Lower Asymptote.
C = X value corresponding to the Y value that is half way between A and D.

Figure C.1 Four parameter logistics curve

In Figure C.1, the asymptotes D and A are the upper and lower limits of the model. An asymptote is a value that the function never reaches. Therefore, the value of a function at or beyond an asymptote cannot be predicted. If MFI $\leq$ A or MFI $\geq$ D, the MFI value is out of the calculable range of the four parameter logistics model. It is not possible to mathematically extrapolate values that are equal to or beyond the asymptote values.

For example, imagine the function F(x) = Log(x). The vertical asymptote for this function is the line x = 0. This means the function

can never reach the line where x= 0. Try calculating Log(0) to see what happens.

In Figure C.1, B is the slope at the inflection point. It is the speed of the function as it moves away from the inflection point. C is the most interesting parameter since it corresponds to the x value that is associated with the y value at the midpoint between the minimum and maximum limits of the function. In a biochemical assay, C corresponds to the concentration (since it is on the x-axis) that produces a 50% response. In drug discovery terms, it is also known as ED50, the 50% effective dosage. It can also be interpreted as LD50 if this dosage kills 50% of the sample population.

C.2
Five Parameter Logistic Curve

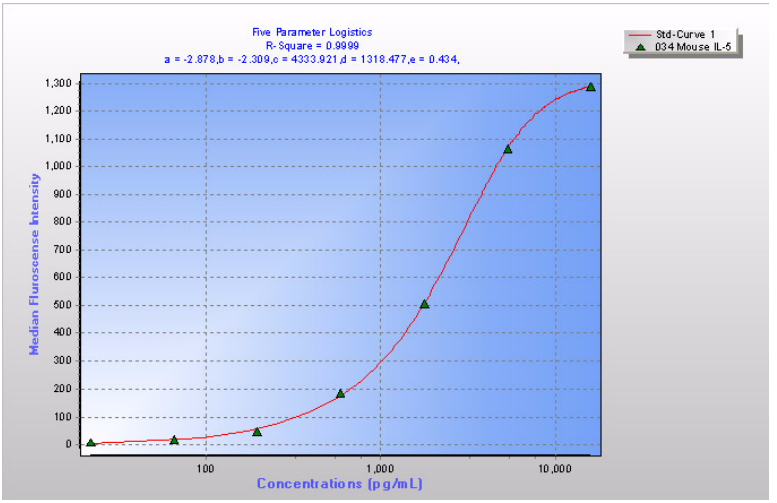


Figure C.2 Five parameter logistic curve

A five parameter logistic curve introduces an additional parameter to the four parameter logistic model to compensate for asymmetric data. In Figure C.2, we can see that the curve is not symmetric because the bottom part of the curve behaves quite differently from the top portion. The additional parameter E compensates for this behavior and corrects for asymmetry.

Fixing Parameter A to Zero

If an assay contains background or blank wells, readings from these wells can be considered noise in the detection. By deducting this noise from the MFI values we can obtain more accurate responses. If we subtract a reasonable background value, then we can further assume that the lowest detectable value should now be zero. Since parameter A represents the lowest detectable concentration value, it can be set to zero.

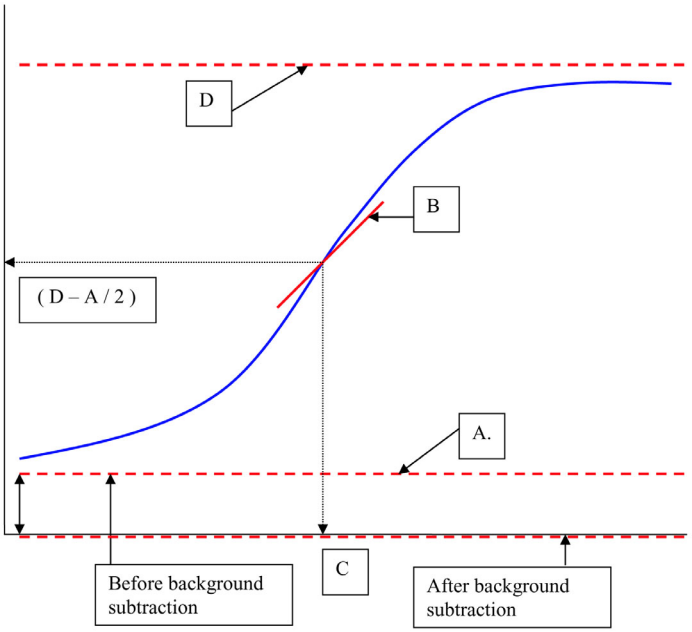


Figure C.1 Five parameter logistics curve

Log-Log Model

The Log-Log model transforms the data to log scale for the x and y values. It applies linear regression to fit a straight line through these points. This model is appropriate for data that are intrinsically linear.

C.3
Heteroscedasticity

Fitting nonlinear models to observed data is often complicated by nonconstant or heterogeneous variability. Heterogeneous variability or *heteroscedasticity* occurs in most types of observed data. This is especially true for biochemical assays where concentration or dose is the predictor and response is often based on count. Therefore, we can expect that measurement error varies with respect to the mean. In the Luminex 100 system, MFI (median fluorescence intensity) values are based on bead counts and vary with the concentration. In this case, we expect the error in detecting MFI values to increase as concentration increases. This is best seen in Figure C.3, a residual plot from a Luminex 100 cytokines assay.

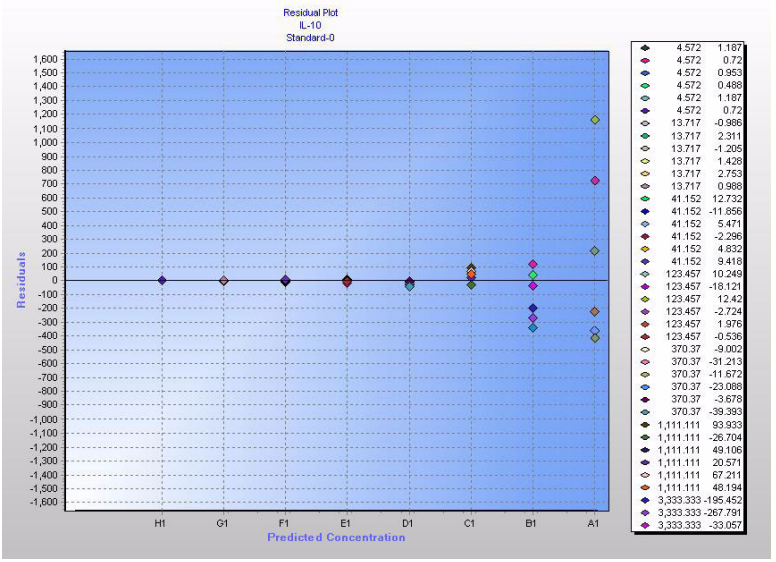


Figure C.3 Residual plot

A residual plot is a graphical representation of how far away an observed concentration is from its expected value. It plots residuals against observed concentrations. In Figure C.3, we can see that the deviation of the observed concentration from the expected value increases as concentration increases. This means the variability is not constant.

Residual plots help you detect nonconstant variability as well as outliers. If a residual plot exhibits data points in a wedge or funnel-shaped pattern, then we can expect the underlying data to have nonconstant variability.

Nonconstant variability complicates curve fitting because the regression process assumes the errors are constant across all data points. When the data violate this assumption, the resulting curve fit is less than optimal. This is illustrated in Figure C.4.

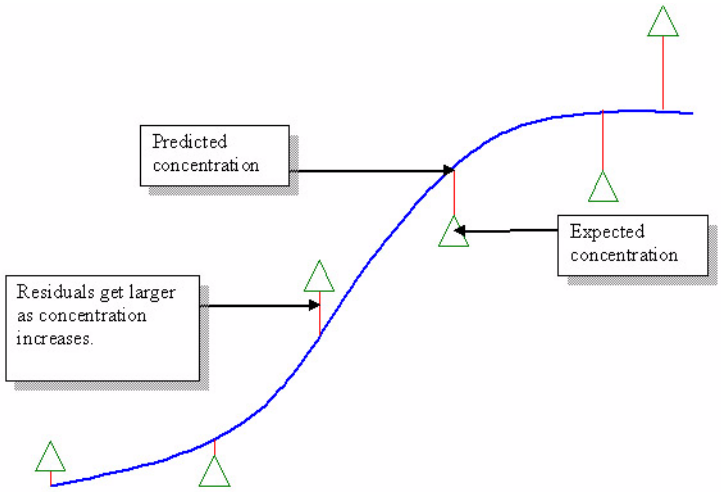


Figure C.4 Data with nonconstant variability
The residual increases as concentration increases.

When we fit a model to data, the curve is applied to all of the data points as closely as possible so that the distances between the predicted and expected concentrations are minimized. In Figure C.4 we can think of the lines that represent the residuals as ropes and each data point as a wrench. Curve fitting can be imagined as pulling the curve line so that it is as close to each point as possible without snapping the actual curve. The best curve fit is reached when the curve is pulled as close as possible to each data point without breaking the actual curve model.

The nonlinear least square algorithm accomplishes this task. The nonlinear (or linear) least square algorithm assumes that all points have the same variability, so all points influence the curve fit equally. However, data that exhibit nonconstant variability violate this assumption. As a result, data points with greater variability assert more

'pull' on the curve. Data points at higher concentrations have more variability then those at lower concentrations, and have greater influence on the curve fit than the points at a lower concentration. As a result, accuracy or sensitivity at the lower concentrations decreases.

C.4
Weighted Nonlinear Least Square

The weighted nonlinear least square method of curve fitting is one way to correct for nonconstant variability. In this method, weights are assigned to each point so that all points have equal influence on the curve. Instead of minimizing the residuals, the method minimizes residuals based on the weight at each point. In mathematical terms, the nonconstant variability is made constant again by these additional weighting factors. If the weight for a point is higher, it will influence the curve fit more. The weight is the inverse of the variance, so points with low variability have more influence during curve fitting, which seems logical. As a result, the curve fit represents the data better and the sensitivity often increases at lower concentrations.

Weighting By 1/Y2

We almost never know what the weight should be at each point, so it must be estimated. One of the most widely used estimation methods is based on a working hypothesis that variance (variability) depends on the square of the mean (the y value). This implies that the standard deviation of a detected MFI value for a particular concentration is directly proportional to the expected concentration. In other words, standard deviation is proportional to the mean.

$\sigma = \mu$

σ = [Some Constant] * μ

σ / μ = [Some Constant]

where σ = standard deviation and μ = mean

Since standard deviation/mean is the coefficient of variation (CV), we can now expect CV to be constant for all points. In this scheme, weight (W) can be estimated using the following expressions:

W = 1/Variance

$W = 1/\sigma^2$

where weight is the inverse of the variance and variance = standard deviation^2

Since we assume, $\sigma \propto \mu$:

$$W \cong 1/\ Y^2$$

(Note that μ = Mean = F(x) = Y)

where W is the estimated weight and Y = F(x) and F(x) is the mean function which is represented by the equation of the model.

In this scheme, we can think of minimizing the residuals based on their relative MFI values. This method is appropriate when higher MFI values have larger residuals than lower MFI values. The wedge shape pattern of data on a residual plot usually confirms this.

Weighting by 1/Y

Another weighting scheme is 1/Y. This method is useful when the MFI values follow a poisson distribution. In a poisson distribution, the standard error of the MFI values is equal to the square root of that value. Since weights are inverses of variances we have:

W = 1/Variance

$W = 1/\sigma^2$

$$W = 1/\ (\sqrt{Y})^2$$

$$W \cong 1/\ Y$$

where:

weight (W) is the inverse of variance

variance = (standard deviation)^2

$\sigma = \sqrt{Y}$

$Y = MFI$

This scheme works well with most Luminex 100 assays. It is a compromise between minimizing absolute residuals and minimizing residuals based on their relative intensity values. If the 1/Y^2 scheme is too strong for a particular data set, this scheme should be tried even if the underlying data distribution is not understood.

Weighting by 1/X and 1/X2

These schemes are rarely used. They give more weight to a data point that is closer to the left side of the graph. A data point on the right side of the graph receives less weight.

Weighting by 1/Stdev^2

If the distribution of MFI values for a particular assay type is understood, we can derive standard errors for the values. In this case, the weights can be calculated exactly as 1/Stdev^2. You can enter the standard deviations into the well grid under the "Standard Deviation" data type and MasterPlex™ QT will automatically calculate the weights.

C.5
Results of Weighting

In addition to the R-Square value, MasterPlex™ QT computes % Recovery values that measure how well the calibration curve (standard curve) fits the observed data.

% Recovery = Observed Concentration/Expected Concentration) * 100

The closer % Recovery is to 100, the better the curve fit at that point. If % Recovery is less than 100, the point is below the curve. If it is greater than 100, the point is above the curve.

Figure C.5 and Figure C.6 show the % Recovery for two different standard curve fits. The standard data in Figure C.6 is from a four parameter logistic equation with no weighting. Note that the % Recovery for H1 is poor. This means the sensitivity deteriorates significantly at lower concentrations.

The data in Figure C.6 is from the same equation with 1/Y weighting. After we apply weighting, the % Recovery for G1 and H1 improves significantly.

Standard Curve Chart | Residual Plot | Standard Data

	Well	Sample Name	Outliers	MFI	Calculated	Expected	Residuals	% Recovery	Background
7	H1	8		55.00	2.12	29.08	-26.96	7.30	68.00
6	G1	7		197.00	84.81	87.24	-2.44	97.21	68.00
5	F1	6		516.00	273.63	261.73	11.90	104.55	68.00
4	E1	5		1406.50	864.88	785.19	79.70	110.15	68.00
3	D1	4		2912.00	2199.04	2355.56	-156.52	93.36	68.00
2	C1	3		5704.00	7345.53	7066.67	278.86	103.95	68.00
1	B1	2		7724.00	20793.86	21200.00	-406.14	98.08	68.00

Figure C.5 Example standard curve data

Four parameter logistics equation fitted to standard data with no weighting.

Standard Curve Chart | Residual Plot | Standard Data

	Well	Sample Name	Outliers	MFI	Calculated	Expected	Residuals	% Recovery	Background
7	H1	8		55.00	27.92	29.08	-1.16	96.00	68.00
6	G1	7		197.00	92.56	87.24	5.32	106.10	68.00
5	F1	6		516.00	256.86	261.73	-4.87	98.14	68.00
4	E1	5		1406.50	822.09	785.19	36.90	104.70	68.00
3	D1	4		2912.00	2192.21	2355.56	-163.35	93.07	68.00
2	C1	3		5704.00	7625.68	7066.67	559.01	107.91	68.00
1	B1	2		7724.00	20322.65	21200.00	-877.35	95.86	68.00

Figure C.6 Example standard curve data

Fitting the four parameter logistics equation to standard data using 1/Y weighting improves the % Recovery at lower concentrations.

APPENDIX D DOSE-RESPONSE ANALYSIS

This appendix provides an introduction to Dose-Response analysis.

Dose-Response Analysis

MFI values are raw response values measured by the Luminex™ 100. Based on these measured responses, standard curves are used to approximate the actual analyte concentration in a sample. Analyte concentrations interpolated from a standard curve are quantified responses. Quantified responses can be used to study dependent variables inherent to a biological or chemical process.

For example, suppose a chemical agent that is given to mice causes an increase in the level of certain cytokines. The chemical agent dosage is the independent variable, and the cytokine concentration is the dependent variable. To understand the potency of the agent, you can perform a Dose-Response analysis. The Dose-Response analysis provides information on the relationship between the agent and changes in the level of cytokines.

Dosages

To conduct a Dose-Response analysis, different dosages of the chemical, biological, or other agent are prepared. For example, eight serial dilutions of an agent (mg/ml) could be prepared and given to eight different mice. A blood sample taken from each mouse is then used to perform a cytokine multiplex assay on the Luminex 100.

Responses

Using MasterPlex QT, you can interpolate the cytokine concentrations of the blood samples from the standard curves of the reagent kit. Now you have both dose and quantified response data (mg/ml).

Regression Analysis

The software uses regression analysis to fit a dose response model to the data points: x = dose and y = response. After the model is fitted, the software provides the LogEC50. Take the antilog of the logEC50 to determine the EC50, the agent dosage that elicits 50% of the response. If 50% of the test subjects die at the EC50 value, the EC50 value is the LD50 value. In this way, you can determine the effectiveness or the toxicity of an agent.

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